

Therapeutic binding molecules

Field of the Invention

5 The present invention relates to organic compounds, such as to binding molecules against CD45 antigen isoforms, such as for example monoclonal antibodies (mAbs).

Background of the Invention

10 One approach in the treatment of a variety of diseases is to achieve the elimination or the inactivation of pathogenic leukocytes and the potential for induction of tolerance to inactivate pathological immune responses.

15 Organ, cell and tissue transplant rejection and the various autoimmune diseases are thought to be primarily the result of T-cell mediated immune response triggered by helper T-cells which are capable of recognizing specific antigens which are captured, processed and presented to the helper T cells by antigen presenting cell (APC) such as macrophages and dendritic cells, in the form of an antigen-MHC complex, i.e. the helper T-cell when recognizing specific antigens is stimulated to produce cytokines such as IL-2 and to express or upregulate some cytokine receptors and other activation molecules and to proliferate.

20 Some of these activated helper T-cells may act directly or indirectly, i.e. assisting effector cytotoxic T-cells or B cells, to destroy cells or tissues expressing the selected antigen. After the termination of the immune response some of the mature clonally selected cells remain as memory helper and memory cytotoxic T-cells, which circulate in the body and rapidly recognize the antigen if appearing again. If the antigen triggering this response is an

25 innocuous environmental antigen the result is allergy, if the antigen is not a foreign antigen, but a self antigen, it can result is autoimmune disease; if the antigen is an antigen from a transplanted organ, the result can be graft rejection.

30 The immune system has developed to recognize self from non-self. This property enables an organism to survive in an environment exposed to the daily challenges of pathogens. This specificity for non-self and tolerance towards self arises during the development of the T cell repertoire in the thymus through processes of positive and negative selection, which also comprise the recognition and elimination of autoreactive T cells. This type of tolerance is

referred to as central tolerance. However, some of these autoreactive cells escape this selective mechanism and pose a potential hazard for the development of autoimmune diseases. To control the autoreactive T cells that have escaped to the periphery, the immune system has peripheral regulatory mechanisms that provide protection against autoimmunity.

5 These mechanisms are a basis for peripheral tolerance.

Cell surface antigens recognized by specific mAbs are generally designated by a CD (Cluster of Differentiation) number assigned by successive International Leukocyte Typing workshops and the term CD45 applied herein refers to the cell surface leukocyte common
10 antigen CD45; and an mAb to that antigen is designated herein as "anti-CD45".

Antibodies against the leukocyte common antigen (LCA) or CD45 are a major component of anti-lymphocyte globulin (ALG). CD45 belongs to the family of transmembrane tyrosine phosphatases and is both a positive and negative regulator of cell activation, depending
15 upon receptor interaction. The phosphatase activity of CD45 appears to be required for activation of Src-family kinases associated with antigen receptor of B and T lymphocytes (Trowbridge IS et al, Annu Rev Immunol. 1994;12:85-116). Thus, in T cell activation, CD45 is essential for signal 1 and CD45-deficient cells have profound defects in TCR-mediated activation events.

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The CD45 antigen exists in different isoforms comprising a family of transmembrane glycoproteins. Distinct isoforms of CD45 differ in their extracellular domain structure which arise from alternative splicing of 3 variable exons coding for part of the CD45 extracellular region (Streuli MF. et al, J. Exp. Med. 1987; 166:1548-1566). The various isoforms of CD45
25 have different extra-cellular domains, but have the same transmembrane and cytoplasmic segments having two homologous, highly conserved phosphatase domains of approximately 300 residues. Different isoform combinations are differentially expressed on subpopulations of T and B lymphocytes (Thomas ML. et al, Immunol. Today 1988; 9:320-325). Some monoclonal antibodies recognize an epitope common to all the different isoforms, while other
30 mAbs have a restricted (CD45R) specificity, dependent on which of the alternatively spliced exons (A, B or C) they recognize. For example, monoclonal antibodies recognizing the product of exon A are consequently designated CD45RA, those recognizing the various isoforms containing exon B have been designated CD45RB (Beverley PCL et al, Immunol. Supp. 1988; 1:3-5). Antibodies such as UCHL1 selectively bind to the 180 kDa isoform

CD45RO (without any of the variable exons A, B or C) which appears to be restricted to a subset of activated T cells, memory cells and cortical thymocytes and is not detected on B cells (Terry LA et al, Immunol. 1988; 64:331-336).

5 **Description of the Figures**

Figure 1 shows that the inhibition of primary MLR by the "candidate mAb" is dose-dependent in the range of 0.001 and 10 µg/ml. "Concentration" is concentration of the "candidate mAb".

10 Figure 2 shows the plasmid map of the expression vector HCMV-G1 HuAb-VHQ comprising the heavy chain having the nucleotide sequence SEQ ID NO:12 (3921-4274) in the complete expression vector nucleotide sequence SEQ ID NO:15.

Figure 3 shows the plasmid map of the expression vector HCMV-G1 HuAb-VHE comprising the heavy chain having the nucleotide sequence SEQ ID NO:11 (3921-4274) in the complete expression vector nucleotide sequence SEQ ID NO:16.

15 Figure 4 shows the plasmid map of the expression vector HCMV-K HuAb-humV1 comprising the light chain having the nucleotide sequence SEQ ID NO:14 (3964-4284) in the complete expression vector nucleotide sequence SEQ ID NO:17.

Figure 5 shows the plasmid map of the expression vector HCMV-K HuAb-humV2 comprising the light chain having the nucleotide sequence SEQ ID NO:13 (3926-4246) in the complete
20 expression vector nucleotide sequence SEQ ID NO:18.

Description of the Invention

We have now found a binding molecule which comprises a polypeptide sequence which
25 binds to CD45RO and CD45RB, hereinafter also designated as a "CD45RO/RB binding molecule". These binding molecule according to the invention may induce immunosuppression, inhibit primary T cell responses and induce T cell tolerance.
Furthermore, the binding molecules of the invention inhibit primary mixed lymphocyte responses (MLR). Cells derived from cultures treated with CD45RO/RB binding molecules
30 preferredly also have impaired proliferative responses in secondary MLR even in the absence of CD45RO/RB binding molecules in the secondary MLR. Such impaired proliferative responses in secondary MLR are an indication of the ability of binding molecules of the invention to induce tolerance.

Furthermore, it is found that *in vivo* administration of CD45RO/RB binding molecule to severe combined immunodeficiency (SCID) mice undergoing xeno-GVHD following injection with human PBMC may prolong mice survival, compared to control treated mice, even though circulating human T cells may still be detected in CD45RO/RB binding molecule treated mice. CD45RB/RO binding molecule may also suppress the inflammatory process that mediates human allograft skin rejection.

By "CD45RO/RB binding molecule" is meant any molecule capable of binding specifically to the CD45RB and CD45RO isoforms of the CD45 antigen, either alone or associated with other molecules. The binding reaction may be shown by standard methods (qualitative assay) including for example any kind of binding assay such as direct or indirect immunofluorescence together with fluorescence microscopy or cytofluorimetric (FACS) analysis, enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay in which binding of the molecule to cells expressing a particular CD45 isoform can be visualized. In addition, the binding of this molecule may result in the alteration of the function of the cells expressing these isoforms. For example inhibition of primary or secondary mixed lymphocyte response (MLR) may be determined, such as an *in vitro* assay or a bioassay for determining the inhibition of primary or secondary MLR in the presence and in the absence of a CD45RO/RB binding molecule and determining the differences in primary MLR inhibition.

Alternatively, the *in vitro* functional modulatory effects can also be determined by measuring the PBMC or T cells or CD4⁺ T cells proliferation, production of cytokines, change in the expression of cell surface molecules e.g. following cell activation in MLR, or following stimulation with specific antigen such as tetanus toxoid or other antigens, or with polyclonal stimulators such as phytohemagglutinin (PHA) or anti-CD3 and anti-CD28 antibodies or phorbol esters and Ca²⁺ ionophores. The cultures are set up in a similar manner as described for MLR except that instead of allogeneic cells as stimulators soluble antigen or polyclonal stimulators such as those mentioned above are used. T cell proliferation is measured preferably as described above by ³H-thymidine incorporation.

Cytokine production is measured preferably by sandwich ELISA where a cytokine capture antibody is coated on the surface of a 96-well plate, the supernatants from the cultures are added and incubated for 1 hr at room temperature and a detecting antibody specific for the particular cytokine is then added, following a second-step antibody conjugated to an enzyme

such as Horseradish peroxidase followed by the corresponding substrate and the absorbance is measured in a plate reader. The change in cell surface molecules may be preferably measured by direct or indirect immunofluorescence after staining the target cells with antibodies specific for a particular cell surface molecule. The antibody can be either
5 directly labeled with flouochrome or a fluorescently labeled second step antibody specific for the first antibody can be used, and the cells are analysed with a cytofluorimeter.

The binding molecule of the invention has a binding specificity for both CD45RO and CD45RB ("CD45RB/RO binding molecule").

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Preferably the binding molecule binds to CD45RO isoforms with a dissociation constant (K_d) $<20\text{nM}$, preferably with a $K_d <15\text{nM}$ or $<10\text{nM}$, more preferably with a $K_d <5\text{nM}$. Preferably the binding molecule binds to CD45RB isoforms with a $K_d <50\text{nM}$, preferably with a $K_d <15\text{nM}$ or $<10\text{nM}$, more preferably with a $K_d <5\text{nM}$.

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In a further preferred embodiment the binding molecule of the invention binds those CD45 isoforms which

- 1) include the A and B epitopes but not the C epitope of the CD45 molecule; and/or
- 2) include the B epitope but not the A and not the C epitope of the CD45 molecule; and/or
- 20 3) do not include any of the A, B or C epitopes of the CD45 molecule.

In yet a further preferred embodiment the binding molecule of the invention does not bind CD45 isoforms which include

- 1) all of the the A, B and C epitopes of the CD45 molecule; and/or
- 25 2) both the B and C epitopes but not the A epitope of the CD45 molecule.

In further preferred embodiments the binding molecule of the invention further

- 1) recognises memory and in vivo alloactivated T cells; and/or
- 2) binds to its target on human T cells, such as for example PEER cells; wherein said
30 binding preferably is with a $K_d <15\text{nM}$, more preferably with a $K_d <10\text{nM}$, most preferably with a $K_d <5\text{nM}$; and/or

- 3) inhibits in vitro alloreactive T cell function, preferably with an IC_{50} of about less than 100nM, preferably less than 50nM or 30nM, more preferably with an IC_{50} of about 10 or 5nM, most preferably with an IC_{50} of about 0,5nM or even 0,1nM; and/or
- 4) induces cell death through apoptosis in human T lymphocytes; and/or
- 5 5) induces alloantigen-specific T cell tolerance in vitro; and/or
- 6) prevents lethal xenogeneic graft versus host disease (GvHD) induced in SCID mice by injection of human PBMC when administered in an effective amount; and/or
- 7) binds to T lymphocytes, monocytes, stem cells, natural killer cells and/or granulocytes, but not to platelets or B lymphocytes; and/or
- 10 8) supports the differentiation of T cells with a characteristic T regulatory cell (Treg) phenotype; and/or
- 9) induces T regulatory cells capable of suppressing naïve T cell activation; and/or
- 10) suppresses the inflammatory process that mediates human allograft skin rejection, in particular, suppresses the inflammatory process that mediates human allograft skin rejection
- 15 *in vivo* in SCID mice transplanted with human skin and engrafted with mononuclear splenocytes.

In a further preferred embodiment the binding molecule of the invention binds to the same epitope as the monoclonal antibody "A6" as described by Aversa et al., Cellular Immunology
20 158, 314-328 (1994).

Due to the above-described binding properties and biological activities, such binding molecules of the invention are particularly useful in medicine, for therapy and/or prophylaxis. Diseases in which binding molecules of the invention are particularly useful include
25 autoimmune diseases, transplant rejection, psoriasis, inflammatory bowel disease and allergies, as will be further set out below.

We have found that a molecule comprising a polypeptide of SEQ ID NO: 1 and a polypeptide of SEQ ID NO: 2 is a CD45RO/RB binding molecule. We also have found the hypervariable
30 regions CDR1', CDR2' and CDR3' in a CD45RO/RB binding molecule of SEQ ID NO:1, CDR1' having the amino acid sequence Arg-Ala-Ser-Gln-Asn-Ile-Gly-Thr-Ser-Ile-Gln (RASQNIGTSIQ), CDR2' having the amino acid sequence Ser-Ser-Ser-Glu-Ser-Ile-Ser (SSSESIS) and CDR3' having the amino acid sequence Gln-Gln-Ser-Asn-Thr-Trp-Pro-Phe-Thr (QQSNTWPFT).

We also have found the hypervariable regions CDR1, CDR2 and CDR3 in a CD45RO/RB binding molecule of SEQ ID NO:2, CDR1 having the amino acid sequence Asn-Tyr-Ile-Ile-His (NYIIH), CDR2 having the amino acid sequence Tyr-Phe-Asn-Pro-Tyr-Asn-His-Gly-Thr-Lys-Tyr-Asn-Glu-Lys-Phe -Lys-Gly (YFNPYNHGTTYNEKFKG) and CDR3 having the amino acid sequence Ser-Gly-Pro-Tyr-Ala-Trp-Phe-Asp-Thr (SGPYAWFDT).

CDRs are 3 specific complementary determining regions which are also called hypervariable regions which essentially determine the antigen binding characteristics. These CDRs are part of the variable region, e.g. of SEQ ID NO: 1 or SEQ ID NO: 2, respectively, wherein these CDRs alternate with framework regions (FR's) e.g. constant regions. A SEQ ID NO: 1 is part of a light chain, e.g. of SEQ ID NO: 3, and a SEQ ID NO:2 is part of a heavy chain, e.g. of SEQ ID NO: 4, in a chimeric antibody according to the present invention. The CDRs of a heavy chain together with the CDRs of an associated light chain essentially constitute the antigen binding site of a molecule of the present invention. It is known that the contribution made by a light chain variable region to the energetics of binding is small compared to that made by the associated heavy chain variable region and that isolated heavy chain variable regions have an antigen binding activity on their own. Such molecules are commonly referred to as single domain antibodies.

In one aspect the present invention provides a molecule comprising at least one antigen binding site, e.g. a CD45RO/RB binding molecule, comprising in sequence the hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence Asn-Tyr-Ile-Ile-His (NYIIH), said CDR2 having the amino acid sequence Tyr-Phe-Asn-Pro-Tyr-Asn-His-Gly-Thr-Lys-Tyr-Asn-Glu-Lys-Phe -Lys-Gly (YFNPYNHGTTYNEKFKG) and said CDR3 having the amino acid sequence Ser-Gly-Pro-Tyr-Ala-Trp-Phe-Asp-Thr (SGPYAWFDT); e.g. and direct equivalents thereof.

In another aspect the present invention provides a molecule comprising at least one antigen binding site, e.g. a CD45RO/RB binding molecule, comprising

- a) a first domain comprising in sequence the hypervariable regions CDR1, CDR2 and CDR3, said CDR1 having the amino acid sequence Asn-Tyr-Ile-Ile-His (NYIIH), said CDR2 having the amino acid sequence Tyr-Phe-Asn-Pro-Tyr-Asn-His-Gly-Thr-Lys-Tyr-Asn-Glu-

- Lys-Phe -Lys-Gly (YFNPYNHGTTYNEKFKG) and said CDR3 having the amino acid sequence Ser-Gly-Pro-Tyr-Ala-Trp-Phe-Asp-Thr (SGPYAWFDT); and
- b) a second domain comprising in sequence the hypervariable regions CDR1', CDR2' and CDR3', CDR1' having the amino acid sequence Arg-Ala-Ser-Gln-Asn-Ile-Gly-Thr-Ser-Ile-Gln (RASQNIGTSIQ), CDR2' having the amino acid sequence Ser-Ser-Ser-Glu-Ser-Ile-Ser (SSSESIS) and CDR3' having the amino acid sequence Gln-Gln-Ser-Asn-Thr-Trp-Pro-Phe-Thr (QQSNTWPFT),
e.g. and direct equivalents thereof.
- 10 In a preferred embodiment the first domain comprising in sequence the hypervariable regions CDR1, CDR2 and CDR3 is an immunoglobulin heavy chain, and the second domain comprising in sequence the hypervariable regions CDR1', CDR2' and CDR3' is an immunoglobulin light chain.
- 15 In another aspect the present invention provides a molecule, e.g. a CD45RO/RB binding molecule, comprising a polypeptide of SEQ ID NO: 1 and/or a polypeptide of SEQ ID NO: 2, preferably comprising in one domain a polypeptide of SEQ ID NO: 1 and in another domain a polypeptide of SEQ ID NO: 2, e.g. a chimeric monoclonal antibody, and in another aspect A molecule, e.g. a CD45RO/RB binding molecule, comprising a polypeptide of SEQ ID NO: 3 and/or a polypeptide of SEQ ID NO: 4, preferably comprising in one domain a polypeptide of SEQ ID NO: 3 and in another domain a polypeptide of SEQ ID NO: 4, e.g. a chimeric monoclonal antibody.
- 25 When the antigen binding site comprises both the first and second domains or a polypeptide of SEQ ID NO: 1 or SEQ ID NO:3, respectively, and a polypeptide of SEQ ID NO: 2 or of SEQ ID NO:4, respectively, these may be located on the same polypeptide, or, preferably each domain may be on a different chain, e.g. the first domain being part of an heavy chain, e.g. immunoglobulin heavy chain, or fragment thereof and the second domain being part of a light chain, e.g. an immunoglobulin light chain or fragment thereof.
- 30 We have further found that a CD45RO/RB binding molecule according to the present invention is a CD45RO/RB binding molecule in mammalian, e.g. human, body environment. A CD45RO/RB binding molecule according to the present invention can thus be designated as a monoclonal antibody (mAb), wherein the binding activity is determined mainly by the

CDR regions as described above, e.g. said CDR regions being associated with other molecules without binding specificity, such as framework, e.g. constant regions, which are substantially of human origin.

- 5 In another aspect the present invention provides a CD45RO/RB binding molecule which is not the monoclonal antibody "A6" as described by Aversa et al., Cellular Immunology 158, 314-328 (1994), which is incorporated by reference for the passages characterizing A6.

10 In another aspect the present invention provides a CD45RO/RB binding molecule according to the present invention which is a chimeric, a humanised or a fully human monoclonal antibody.

Examples of a CD45RO/RB binding molecules include chimeric or humanised antibodies e.g. derived from antibodies as produced by B-cells or hybridomas and or any fragment thereof, e.g. F(ab')₂ and Fab fragments, as well as single chain or single domain antibodies. A single chain antibody consists of the variable regions of antibody heavy and light chains covalently bound by a peptide linker, usually consisting of from 10 to 30 amino acids, preferably from 15 to 25 amino acids. Therefore, such a structure does not include the constant part of the heavy and light chains and it is believed that the small peptide spacer should be less antigenic than a whole constant part. By a chimeric antibody is meant an antibody in which the constant regions of heavy and light chains or both are of human origin while the variable domains of both heavy and light chains are of non-human (e.g. murine) origin. By a humanised antibody is meant an antibody in which the hypervariable regions (CDRs) are of non-human (e.g. murine) origin while all or substantially all the other part, e.g. the constant regions and the highly conserved parts of the variable regions are of human origins. A humanised antibody may however retain a few amino acids of the murine sequence in the parts of the variable regions adjacent to the hypervariable regions.

30 Hypervariable regions, i.e. CDR's according to the present invention may be associated with any kind of framework regions, e.g. constant parts of the light and heavy chains, of human origin. Suitable framework regions are e.g. described in "Sequences of proteins of immunological interest", Kabat, E.A. et al, US department of health and human services, Public health service, National Institute of health. Preferably the constant part of a human heavy chain may be of the IgG1 type, including subtypes, preferably the constant part of a

human light chain may be of the κ or λ type, more preferably of the κ type. A preferred constant part of a heavy chain is a polypeptide of SEQ ID NO: 4 (without the CDR1', CDR2' and CDR3' sequence parts which are specified above) and a preferred constant part of a light chain is a polypeptide of SEQ ID NO: 3 (without the CDR1, CDR2 and CDR3 sequence parts which are specified above).

We also have found a humanised antibody comprising a light chain variable region of amino acid SEQ ID NO:7 or of amino acid SEQ ID NO:8, which comprises CDR1', CDR2' and CDR3' according to the present invention and a heavy chain variable region of SEQ ID NO:9 or of SEQ ID NO:10, which comprises CDR1, CDR2 and CDR3 according to the present invention.

In another aspect the present invention provides a humanised antibody comprising a polypeptide of SEQ ID NO:9 or of SEQ ID NO:10 and a polypeptide of SEQ ID NO:7 or of SEQ ID NO:8.

In another aspect the present invention provides a humanised antibody comprising

- a polypeptide of SEQ ID NO:9 and a polypeptide of SEQ ID NO:7,
- a polypeptide of SEQ ID NO:9 and a polypeptide of SEQ ID NO:8,
- a polypeptide of SEQ ID NO:10 and a polypeptide of SEQ ID NO:7, or
- a polypeptide of SEQ ID NO:10 and a polypeptide of SEQ ID NO:8.

A polypeptide according to the present invention, e.g. of a herein specified sequence, e.g. of CDR1, CDR2, CDR3, CDR1', CDR2', CDR3', or of a SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10 includes direct equivalents of said (poly)peptide (sequence); e.g. including a functional derivative of said polypeptide. Said functional derivative may include covalent modifications of a specified sequence, and/or said functional derivative may include amino acid sequence variants of a specified sequence.

"Polypeptide", if not otherwise specified herein, includes any peptide or protein comprising amino acids joined to each other by peptide bonds, having an amino acid sequence starting at the N-terminal extremity and ending at the C-terminal extremity. Preferably the polypeptide of the present invention is a monoclonal antibody, more preferred is a chimeric

(V-grafted) or humanised (CDR-grafted) monoclonal antibody. The humanised (CDR-grafted) monoclonal antibody may or may not include further mutations introduced into the framework (FR) sequences of the acceptor antibody.

- 5 A functional derivative of a polypeptide as used herein includes a molecule having a qualitative biological activity in common with a polypeptide to the present invention, i.e. having the ability to bind to CD45RO and CD45RB. A functional derivative includes fragments and peptide analogs of a polypeptide according to the present invention. Fragments comprise regions within the sequence of a polypeptide according to the present
10 invention, e.g. of a specified sequence. The term "derivative" is used to define amino acid sequence variants, and covalent modifications of a polypeptide according to the present invention. e.g. of a specified sequence. The functional derivatives of a polypeptide according to the present invention, e.g. of a specified sequence, preferably have at least about 65%, more preferably at least about 75%, even more preferably at least about 85%, most
15 preferably at least about 95% overall sequence homology with the amino acid sequence of a polypeptide according to the present invention, e.g. of a specified sequence, and substantially retain the ability to bind to CD45RO and CD45RB.

The term "covalent modification" includes modifications of a polypeptide according to the
20 present invention, e.g. of a specified sequence; or a fragment thereof with an organic proteinaceous or non-proteinaceous derivatizing agent, fusions to heterologous polypeptide sequences, and post-translational modifications. Covalent modified polypeptides, e.g. of a specified sequence, still have the ability bind to CD45RO and CD45RB by crosslinking. Covalent modifications are traditionally introduced by reacting targeted amino acid residues
25 with an organic derivatizing agent that is capable of reacting with selected sides or terminal residues, or by harnessing mechanisms of post-translational modifications that function in selected recombinant host cells. Certain post-translational modifications are the result of the action of recombinant host cells on the expressed polypeptide. Glutamyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and
30 aspartyl residues. Alternatively, these residues are deaminated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl, tyrosine or threonyl residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains, see e.g. T. E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco, pp. 79-

86 (1983). Covalent modifications e.g. include fusion proteins comprising a polypeptide according to the present invention, e.g. of a specified sequence and their amino acid sequence variants, such as immunoadhesins, and N-terminal fusions to heterologous signal sequences.

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"Homology" with respect to a native polypeptide and its functional derivative is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues of a corresponding native polypeptide, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not
10 considering any conservative substitutions as part of the sequence identity. Neither N- or C-terminal extensions nor insertions shall be construed as reducing identity or homology. Methods and computer programs for the alignment are well known.

"Amino acid(s)" refer to all naturally occurring L- α -amino acids, e.g. and including D-amino acids. The amino acids are identified by either the well known single-letter or three-letter
15 designations.

The term "amino acid sequence variant" refers to molecules with some differences in their amino acid sequences as compared to a polypeptide according to the present invention, e.g. of a specified sequence. Amino acid sequence variants of a polypeptide according to the
20 present invention, e.g. of a specified sequence, still have the ability to bind to CD45RO and CD45RB. Substitutional variants are those that have at least one amino acid residue removed and a different amino acid inserted in its place at the same position in a polypeptide according to the present invention, e.g. of a specified sequence. These substitutions may be single, where only one amino acid in the molecule has been substituted, or they may be
25 multiple, where two or more amino acids have been substituted in the same molecule. Insertional variants are those with one or more amino acids inserted immediately adjacent to an amino acid at a particular position in a polypeptide according to the present invention, e.g. of a specified sequence. Immediately adjacent to an amino acid means connected to either the α -carboxy or α -amino functional group of the amino acid. Deletional variants are those
30 with one or more amino acids in a polypeptide according to the present invention, e.g. of a specified sequence, removed. Ordinarily, deletional variants will have one or two amino acids deleted in a particular region of the molecule.

We also have found the polynucleotide sequences of

- GGCCAGTCAGAACATTGGCACAAGCATACAGTG, encoding the amino acid sequence of CDR1,
- TTCTTCTGAGTCTATCTCTGG; encoding the amino acid sequence of CDR 2,
- 5 - ACAAAGTAATACCTGGCCATTACGTT encoding the amino acid sequence of CDR 3,
- TTATATTATCCACTG, encoding the amino acid sequence of CDR1',
- TTTTAATCCTTACAATCATGGTACTAAGTACAATGAGAAGTTCAAAGGCAG encoding the amino acid sequence of CDR2',
- AGGACCCTATGCCTGGTTTGACACCTG encoding the amino acid sequence of CDR3',
- 10 - SEQ ID NO:5 encoding a polypeptide of SEQ ID NO: 1, i.e. the variable region of a light chain of an mAb according to the present invention;
- SEQ ID NO:6 encoding a polypeptide of SEQ ID NO:2, i.e. the variable region of the heavy chain of an mAb according to the present invention;
- SEQ ID NO:11 encoding a polypeptide of SEQ ID NO:9. i.e. a heavy chain variable region
- 15 including CDR1, CDR2 and CDR3 according to the present invention;
- SEQ ID NO:12 encoding a polypeptide of SEQ ID NO:10, i.e. a heavy chain variable region including CDR1, CDR2 and CDR3 according to the present invention;
- SEQ ID NO:13 encoding a polypeptide of SEQ ID NO:7, i.e. a light chain variable region including CDR1', CDR2' and CDR3' according to the present invention; and
- 20 - SEQ ID NO:14 encoding a polypeptide of SEQ ID NO:8, i.e. a light chain variable region including CDR1', CDR2' and CDR3' according to the present invention.

In another aspect the present invention provides isolated polynucleotides comprising polynucleotides encoding a CD45RO/RB binding molecule, e.g. encoding the amino acid

25 sequence of CDR1, CDR2 and CDR3 according to the present invention and/or, preferably and, polynucleotides encoding the amino acid sequence of CDR1', CDR2' and CDR3' according to the present invention; and

Polynucleotides comprising a polynucleotide of SEQ ID NO: 5 and/or, preferably and, a polynucleotide of SEQ ID NO: 6; and

- 30 Polynucleotides comprising polynucleotides encoding a polypeptide of SEQ ID NO:7 or SEQ ID NO:8 and a polypeptide of SEQ ID NO:9 or SEQ ID NO:10; e.g. encoding
- a polypeptide of SEQ ID NO:7 and a polypeptide of SEQ ID NO:9,
 - a polypeptide of SEQ ID NO:7 and a polypeptide of SEQ ID NO:10,
 - a polypeptide of SEQ ID NO:8 and a polypeptide of SEQ ID NO:9, or

- a polypeptide of SEQ ID NO:8 and a polypeptide of SEQ ID NO:10; and
 Polynucleotides comprising a polynucleotide of SEQ ID NO:11 or of SEQ ID NO:12 and a
 polynucleotide of SEQ ID NO:13 or a polynucleotide of SEQ ID NO:14, preferably
 comprising

- 5 - a polynucleotide of SEQ ID NO:11 and a polynucleotide of SEQ ID NO:13,
 - a polynucleotide of SEQ ID NO:11 and a polynucleotide of SEQ ID NO:14,
 - a polynucleotide of SEQ ID NO:12 and a polynucleotide of SEQ ID NO:13, or
 - a polynucleotide of SEQ ID NO:12 and a polynucleotide of SEQ ID NO:14.

10 “Polynucleotide”, if not otherwise specified herein, includes any polyribonucleotide or
 polydeoxyribonucleotide, which may be unmodified RNA or DNA, or modified RNA or DNA,
 including without limitation single and double stranded RNA, and RNA that is a mixture of
 single- and double-stranded regions.

15 A polynucleotide according to the present invention, e.g. a polynucleotide encoding the
 amino acid sequence CDR1, CDR2, CDR3, CDR1', CDR2', CDR3', or of SEQ ID NO:1, SEQ
 ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 or SEQ
 ID NO:10, respectively, such as a polynucleotide of SEQ ID NO:5, SEQ ID NO:6, SEQ ID
 NO:11, SEQ ID NO:12, SEQ ID NO:13 or SEQ ID NO:14, respectively, includes allelic
 20 variants thereof and/or their complements; e.g. including a polynucleotide that hybridizes to
 the nucleotide sequence of SEQ ID NO: 5, SEQ ID NO:6, SEQ ID NO:11, SEQ ID NO:12,
 SEQ ID NO:13 or SEQ ID NO:14, respectively; e.g. encoding a polypeptide having at least
 80% identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:7,
 SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10, respectively, e.g. including a functional
 25 derivative of said polypeptide, e.g. said functional derivative having at least 65% homology
 with SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:7, SEQ ID
 NO:8, SEQ ID NO:9 or SEQ ID NO:10, respectively, e.g. said functional derivative including
 covalent modifications of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID
 NO:7, SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10, respectively, e.g. said functional
 30 derivative including amino acid sequence variants of SEQ ID NO:1, SEQ ID NO:2, SEQ ID
 NO:3, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10,
 respectively; e.g. a SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:11, SEQ ID NO:12, SEQ ID
 NO:13 or SEQ ID NO:14, respectively includes a sequence, which as a result of the
 redundancy (degeneracy) of the genetic code, also encodes a polypeptide of SEQ ID NO:1,

SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10, respectively, or encodes a polypeptide with an amino acid sequence which has at least 80% identity with the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10, respectively.

A CD45RO/RB binding molecule, e.g. which is a chimeric or humanised antibody, may be produced by recombinant DNA techniques. Thus, one or more DNA molecules encoding the CD45RO/RB may be constructed, placed under appropriate control sequences and transferred into a suitable host (organism) for expression by an appropriate vector.

In another aspect the present invention provides a polynucleotide which encodes a single, heavy and/or a light chain of a CD45RO/RB binding molecule according to the present invention; and the use of a polynucleotide according to the present invention for the production of a CD45RO/RB binding molecule according to the present invention by recombinant means.

A CD45RO/RB binding molecule may be obtained according, e.g. analogously, to a method as conventional together with the information provided herein, e.g. with the knowledge of the amino acid sequence of the hypervariable or variable regions and the polynucleotide sequences encoding these regions. A method for constructing a variable domain gene is e.g. described in EP 239 400 and may be briefly summarized as follows: A gene encoding a variable region of a mAb of whatever specificity may be cloned. The DNA segments encoding the framework and hypervariable regions are determined and the DNA segments encoding the hypervariable regions are removed. Double stranded synthetic CDR cassettes are prepared by DNA synthesis according to the CDR and CDR' sequences as specified herein. These cassettes are provided with sticky ends so that they can be ligated at junctions of a desired framework of human origin. Polynucleotides encoding single chain antibodies may also be prepared according to, e.g. analogously, to a method as conventional. A polynucleotide according to the present invention thus prepared may be conveniently transferred into an appropriate expression vector.

Appropriate cell lines may be found according, e.g. analogously, to a method as conventional. Expression vectors, e.g. comprising suitable promotor(s) and genes encoding

heavy and light chain constant parts are known e.g. and are commercially available. Appropriate hosts are known or may be found according, e.g. analogously, to a method as conventional and include cell culture or transgenic animals.

- 5 In another aspect the present invention provides an expression vector comprising a polynucleotide encoding a CD45RO/RB binding molecule according to the present invention, e.g. of sequence SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17 or SEQ ID NO:18.

In another aspect the present invention provides

- 10 - An expression system comprising a polynucleotide according to the present invention wherein said expression system or part thereof is capable of producing a CD45RO/RB binding molecule according to the present invention, when said expression system or part thereof is present in a compatible host cell;
and
15 - An isolated host cell which comprises an expression system as defined above.

We have further found that a CD45RO/RB binding molecule according to the present invention inhibit primary alloimmune responses in a dose-dependent fashion as determined by in vitro MLR. The results indicate that the cells which had been alloactivated in the
20 presence of a CD45RO/RB binding molecule according to the present invention are impaired in their responses to alloantigen. This confirms the indication that a CD45RO/RB binding molecule according to the present invention can act directly on the effector alloreactive T cells and modulate their function. In addition, the functional properties of T cells derived from the primary MLR were further studied in restimulation experiments in secondary MLR, using
25 specific stimulator cells or third-party stimulators to assess the specificity of the observed functional effects. We have found that the cells derived from primary MLRs in which a CD45RO/RB binding molecule according to the present invention is present, were impaired in their ability to respond to subsequent optimal stimulation with specific stimulator cells, although there was no antibody added to the secondary cultures. The specificity of the
30 inhibition was demonstrated by the ability of cells treated with a CD45RO/RB binding molecule according to the present invention to respond normally to stimulator cells from unrelated third-party donors. Restimulation experiments using T cells derived from primary MLR cultures thus indicate that the cells which had been alloactivated a CD45RO/RB binding

molecule according to the present invention are hyporesponsive, i.e. tolerant, to the original alloantigen. Further biological activities are described in examples 7, and 9 to 12.

Furthermore we have found that cell proliferation in cells pre-treated with a CD45RO/RB binding molecule according to the present invention could be rescued by exogenous IL-2. This indicates that treatment of alloreactive T cells with a CD45RO/RB binding molecule according to the present invention induces a state of tolerance. Indeed, the reduced proliferative responses observed in cells treated with a CD45RO/RB binding molecule according to the present invention, was due to impairment of T cell function, and these cells were able to respond to exogenous IL-2, indicating that these cells are in an anergic, true unresponsive state. The specificity of this response was shown by the ability of cells treated with a CD45RO/RB binding molecule according to the present invention to proliferate normally to unrelated donor cells to the level of the control treated cells.

In addition experiments indicate that the binding of a CD45RO/RB binding molecule according to the present invention to CD45RO and CD45RB may inhibit the memory responses of peripheral blood mononuclear cells (PBMC) from immunized donors to specific recall antigen. Binding of a CD45RO/RB binding molecule according to the present invention to CD45RO and CD45RB thus is also effective in inhibiting memory responses to soluble Ag. The ability of a CD45RO/RB binding molecule according to the present invention to inhibit recall responses to tetanus in PBMC from immunized donors indicate that a CD45RO/RB binding molecule according to the present invention is able to target and modulate the activation of memory T cells. E.g. these data indicate that a CD45RO/RB binding molecule according to the present invention in addition to recognizing alloreactive and activated T cells is able to modulate their function, resulting in induction of T cell anergy. This property may be important in treatment of ongoing immune responses to autoantigens and allergens and possibly to alloantigens as seen in autoimmune diseases, allergy and chronic rejection, and diseases, such as psoriasis, inflammatory bowel disease, where memory responses play a role in the maintenance of disease state. It is believed to be an important feature in a disease situation, such as in autoimmune diseases in which memory responses to autoantigens may play a major role for the disease maintenance.

We have also found that a CD45RO/RB binding molecule according to the present invention may modulate T cell proliferative responses in a mixed lymphocyte response (MLR) *in vivo*,

i.e. a CD45RO/RB binding molecule according to the present invention was found to have corresponding inhibitory properties in vivo testing.

A CD45RO/RB binding molecule according to the present invention may thus have

5 immunosuppressive and tolerogenic properties and may be useful for in vivo and ex-vivo tolerance induction to alloantigens, autoantigens, allergens and bacterial flora antigens, e.g. a CD45RO/RB binding molecule according to the present invention may be useful in the treatment and prophylaxis of diseases e.g. including autoimmune diseases, such as, but not limited to, rheumatoid arthritis, autoimmune thyroiditis, Graves disease, type I and type II
10 diabetes, multiple sclerosis, systemic lupus erythematosus, Sjögren syndrome, scleroderma, autoimmune gastritis, glomerulonephritis, transplant rejection, e.g. organ and tissue allograft and xenograft rejection, graft versus host disease (GVHD), and also psoriasis, inflammatory bowel disease and allergies.

15 In another aspect the present invention provides the use of a CD45RO/RB binding molecule according to the present invention as a pharmaceutical, e.g. in the treatment and prophylaxis of autoimmune diseases, transplant rejection, psoriasis, inflammatory bowel disease and allergies.

20 In another aspect the present invention provides a CD45RO/RB binding molecule according to the present invention for the production of a medicament in the treatment and prophylaxis of diseases associated with autoimmune diseases, transplant rejection, psoriasis, inflammatory bowel disease and allergies.

25 In another aspect the present invention provides a pharmaceutical composition comprising a CD45RO/RB binding molecule according to the present invention in association with at least one pharmaceutically acceptable carrier or diluent.

A pharmaceutical composition may comprise further, e.g. active, ingredients, e.g. other
30 immunomodulatory antibodies such as, but not confined to anti-ICOS, anti-CD154, anti-CD134L or recombinant proteins such as, but not confined to rCTLA-4 (CD152), rOX40 (CD134), or immunomodulatory compounds such as, but not confined to cyclosporin A, FTY720, RAD, rapamycin, FK506, 15-deoxyspergualin, steroids.

In another aspect the present invention provides a method of treatment and/or prophylaxis of diseases associated with autoimmune diseases, transplant rejection, psoriasis, inflammatory bowel disease and allergies comprising administering to a subject in need of such treatment and/or prophylaxis an effective amount of a CD45RO/RB binding molecule according to the present invention, e.g. in the form of a pharmaceutical composition according to the present invention.

Autoimmune diseases to be treated with binding molecule of the present invention further include, but are not limited to, rheumatoid arthritis, autoimmune thyroiditis, Graves disease, type I and type II diabetes, multiple sclerosis, systemic lupus erythematosus, Sjögren syndrome, scleroderma, autoimmune gastritis, glomerulonephritis; transplant rejection, e.g. organ and tissue allograft and xenograft rejection and graft-versus-host disease (GVHD).

EXAMPLES

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. In the following examples all temperatures are in degree Celsius.

The "candidate mAb" or "chimeric antibody" is a CD45RO/RB binding molecule according to the present invention comprising light chain of SEQ ID NO:3 and heavy chain of SEQ ID NO:4.

The "humanised antibody" is a CD45RO/RB binding molecule according to the present invention comprising a polypeptide of SEQ ID NO:8 and polypeptide of SEQ ID NO:9 or a polypeptide of SEQ ID NO:8 and a polypeptide of SEQ ID NO:10.

The following abbreviations are used:

APC	antigen presenting cell
c.p.m.	counts per minute
ELISA	enzyme linked immuno-sorbant assay
FACS	fluorescence activated cell sorting

	Fc	fragment crystallizable
	F(ab') ₂	fragment antigen-binding; bivalent
	FITC	fluorescein isothiocyanate
	FBS	foetal bovine serum
5	GVHD	graft-vs-host disease
	HCMV	human cytomegalovirus promoter
	IFN- γ	interferon gamma
	IgE	immunoglobulin isotype E
	IgG	immunoglobulin isotype G
10	IL-2	interleukin-2
	IU	international units
	MLR	mixed lymphocyte reaction
	MLC	mixed lymphocyte culture
	MP1	matrix protein 1 from hemophilus influenza
15	PBS	phosphate-buffered saline
	PBL	peripheral blood leukocytes
	PBMC	peripheral blood mononuclear cells
	PCR	polymerase chain reaction
	SCID	severe combined immunodeficiency
20	T _{reg}	T regulatory cells
	xGVHD	xeno-graft-vs-host disease

Example 1: Primary mixed lymphocyte response (MLR)*Cells*

- 5 Blood samples are obtained from healthy human donors. Peripheral blood mononuclear cells (PBMC) are isolated by centrifugation over Ficoll-Hypaque (Pharmacia LKB) from leukocytes from whole peripheral blood, leukopheresis or buffy coats with known blood type, but unknown HLA type. In some MLR experiments, PBMC are directly used as the stimulator cells after the irradiation at 40 Gy. In the other experiments, T cells were depleted from
- 10 PBMC by using CD2 or CD3 Dynabeads (Dynal, Oslo, Norway). Beads and contaminating cells are removed by magnetic field. T cell-depleted PBMC are used as stimulator cells after the irradiation.

- PBMC, CD3⁺ T cells or CD4⁺ T cells are used as the responder cells in MLR. Cells are prepared from different donors to stimulator cells. CD3⁺ T cells are purified by negative
- 15 selection using anti-CD16 mAb (Zymed, CA), goat anti-mouse IgG Dynabeads, anti-CD14 Dynabeads, CD19 Dynabeads. In addition anti-CD8 Dynabeads are used to purify CD4⁺ T cells. The cells obtained are analyzed by FACScan or FACSCalibur (Becton Dickinson & Co., CA) and the purity of the cells obtained was >75%. Cells are suspended in RPMI1640 medium, supplemented with 10 % heat-inactivated FBS, penicillin, streptomycin and L-
- 20 glutamine.

Reagents

- The chimeric anti-CD45R0/RB mAb "candidate mAb" and an isotype matched control chimeric antibody is also generated. Mouse (Human) control IgG₁ antibody specific for KLH
- 25 (keyhole limpet hemocyanin) or recombinant human IL-10 is purchased from BD Pharmingen (San Diego, CA). Anti-human CD154 mAb 5c8 is according to Lederman et al 1992.

Primary Mixed lymphocyte response (MLR)

- 30 Aliquots of 1×10^5 PBMC or 5×10^4 of CD3⁺ or CD4⁺ cells are mixed with 1×10^5 irradiated PBMC or 5×10^4 T cells-depleted irradiated (50 Gy) PBMC in the each well of 96-well culture plates (Costar, Cambridge, MA) in the presence of the indicated mAb or absence of Ab. In some experiments, F(ab')₂ fragment of goat anti-mouse Ig or goat anti-human Ig specific for

Fc portion (Jackson ImmunoResearch, West Grove, PA) is added at 10 µg/ml in addition to the candidate mAb To ensure optimal in vitro cross-linking of the target CD45 molecules.

The mixed cells are cultured for 4 or 5 days at 37°C in 5% CO₂ and proliferation is determined by pulsing the cells with ³H-thymidine for the last 16 - 20 hours of culture.

- 5 Other experiments are similar to those described above, but with the following exceptions: 1) Medium used is EX-VIVO (Bio-Whittaker) containing 10% FBS and 1% human plasma; 2) Anti-mouse total IgG (5 µg/ml) is used as secondary cross-linking step; 3) Irradiation of stimulator cells is 60 Gy.

- 10 Primary MLR is performed in the presence of the "candidate mAb" or control chimeric IgG₁ (10 µg/ml) both with a second step reagent, F(ab')₂ fragment of goat anti-human Ig specific for Fc portion (10 µg/ml). Percentage inhibition by the "candidate mAb" is calculated in comparison with the cell proliferation in the presence of control IgG₁. Results are shown in TABLE 1 below:

TABLE 1

- 15 Inhibition of primary MLR by 10 µg/ml of a candidate mAb according to the present invention

Responder	Stimulator (Irr. PBMC)	% of Inhibition
#211 CD4	#219 CD3	63.51
#220 CD4	#219 CD3 depl.	63.07
#227 CD4	#220 CD3 depl.	65.96
#229 CD4	#219 CD3 depl.	50.76
Average± SD		60.83 ±6.83 *

* Significantly different from control value (P<0.001)

- 20 A candidate mAb according to the present invention inhibits primary MLR as can be seen from TABLE 1. The average inhibitory effect is 60.83 ± 6.83 % in four different donors-derived CD4⁺ T cells and statistically significant.

The inhibition of primary MLR by the "candidate mAb" is shown to be dose-dependent in the range of 0.001 and 10 µg/ml of the "candidate mAb" as shown in Figure 1.

- 25 The IC₅₀ for the inhibition of primary MLR by a "candidate mAb" is determined from the results of three separate MLR experiments using one donor PBMC as responder cells. Thus, responder CD4⁺ T cells from Donor #229 and #219 and irradiated PBMC depleted of T cells as stimulators are mixed in the presence of a "candidate mAb" or control chimeric Ab with 10 µg/ml of F(ab')₂ fragment of goat anti-human Ig. Experiments are repeated 3 times and percentage of proliferation in the presence of a "candidate mAb" is calculated in comparison

with the T cell proliferation in the presence of control Ab. IC_{50} value is determined using Origin (V. 6.0®). The cellular activity IC_{50} value is calculated to be 0.87 ± 0.35 nM (0.13 ± 0.052 μ g/ml).

5 **Example 2: Secondary MLR**

In order to assess whether a "candidate mAb" induces unresponsiveness of $CD4^+$ T cells to specific alloantigens, secondary MLR is performed in the absence of any antibodies after the primary MLC. $CD4^+$ T cells are cultured with irradiated allogeneic stimulator cells (T cells-depleted PBMC) in the presence of the indicated antibody in 96-well culture plates for 10 days (primary MLC). Then, cells are collected, layered on a Ficoll-Hypaque gradient to remove dead cells, washed twice with RPMI, and restimulated with the same stimulator, 3rd party stimulator cells or IL-2 (50 U/ml). The cells are cultured for 3 days and the proliferative response is determined by pulsing the cells with 3H -thymidine for the last 16 - 20 hours of culture.

Specifically, $CD4^+$ T cells are cultured with irradiated allogeneic stimulator cells (T cells-depleted PBMC taken from other donors) in the presence of 10 μ g/ml of the "candidate mAb", control IgG1 chimeric Ab and F(ab')₂ fragment of goat anti-human Ig. Primary MLR proliferation is determined on day 5. For secondary MLR, the responder and stimulator cells are cultured for 10 days in the presence of the "candidate mAb", then the cells are harvested, washed twice in RPMI1640 and restimulated with specific stimulator, third-party stimulators or IL-2 (50 U/ml) in the absence of any Ab. Cell proliferation is determined on day 3. Results set out in TABLE 2:

TABLE 2

Responder $CD4^+$ T cells Donor #	% Inhibition of 2 nd MLR
#211	49.90*
#220	59.33*
#227	58.68*

* Significantly different from control value ($p < 0.001$ determined by t-test, SigmaStat V.2.03). # $p = 0.046$

In order to test whether the impaired proliferation is due to unresponsiveness as a consequence of the treatment with a "candidate mAb", the cells derived from primary MLR are cultured in the presence of IL-2 (50 U/ml). Addition of IL-2 results in the rescue of

proliferative responses of the T cells which had been treated with a "candidate mAb" in primary MLR, to levels similar to those observed in the presence of IgG₁ control Ab. These data indicate that the impaired secondary response in T cells treated with a "candidate mAb" is due to functional alteration of the responder T cells which become unresponsive to the specific stimulator cells.

Percentage inhibition is calculated according to the following formula:

$$\frac{\text{c.p.m. with control Ab} - \text{c.p.m. with "candidate mAb"}}{\text{c.p.m. with control Ab}} \times 100$$

Statistical analysis is performed using SigmaStat (Vers. 2.03).

The data is analyzed by two-way ANOVA followed by Dunnett method. In all test procedures probabilities <0.05 are considered as significant. In some experiments t-test is used (SigmaStat V.2.03).

Example 3: In vivo survival studies in SCID-mice

Engraftment of hu-PBL in SCID mice

Human peripheral blood mononuclear cells (PBMC) are injected intraperitoneally into SCID mice C.B 17 /Gbm^sTac-Prkdc^{scid} Lys^{tg} mice (Taconic, Germantown, NY) in an amount sufficient to induce a lethal xenogeneic graft-versus-host disease (xGvHD) in >90% of the mice within 4 weeks after cell transfer. Such treated SCID mice are hereinafter designated as hu-PBL-SCID mice

Mab-treatment of hu-PBL-SCID mice

Hu-PBL-SCID mice are treated with a "candidate mAb" or mouse or chimeric isotype matched mAb controls at day 0, immediately after PBMC injection, at day 3, day 7 and at weekly intervals thereafter. Mabs are delivered subcutaneously in 100 µl PBS at a final concentration of 5 mg/kg body weight. The treatment was stopped when all control mice were dead.

Evaluation of treatment results

The main criterion to assess the efficacy of a "candidate mAb" in this study was the survival of the hu-PBL-SCID mice. The significance of the results is evaluated by the statistical

method of survival analysis using the Log-rank test (Mantel method) with the help of the Systat v9.01 software. The method of survival analysis is a non-parametric test, which not only consider whether a particular mouse is still alive but also whether if it was sacrificed for reasons irrelevant to the treatment/disease such as the requirement of perform in vitro analysis with its organs/cells. Biopsies of liver, lung, kidney and spleen are obtained from dead mice for further evaluation. In addition, hu-PBL-SCID mice are weighed at the beginning (before cell transfer) and throughout (every two days) the experiment as an indirect estimation of their health status. Linear regression lines were generated using the body weight versus days post-PBMC transfer values obtained from each mouse and subsequently, their slopes (control versus anti-CD45 treated mice) were compared using the non-parametric Mann-Whitney test.

Results

All hu-PBL-SCID mice treated with mouse mAb controls had infiltrated human leukocytes in the lung, liver and spleen and died (4/4) within ca. 2 to 3 weeks after cell transfer. Death is a likely consequence of xGvHD. Control mAb-treated mice furthermore lost weight in a linear manner, ca. 10% and more within 3 weeks.

All hu-PBL-SCID mice treated with a "candidate mAb" survived (4/4) without any apparent sign of disease more than 4 weeks, even although "candidate mAb"-treatment was stopped after 3 weeks. "Candidate mAb"-treated mice increased weight in a linear manner, up to ca. 5% within 4 weeks.

Example 4: Expression of antibodies of the invention

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Expression of humanised antibody comprising a SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10

Expression vectors according to the plasmid map shown in Figures 2 to 5 are constructed, comprising the corresponding nucleotides encoding the amino acid sequence of humanised light chain variable region humV1 (SEQ ID NO:7), humanised light chain variable region humV2 (SEQ ID NO:8), humanised heavy chain variable region VHE (SEQ ID NO:9), or humanised heavy chain variable region VHQ (SEQ ID NO:10), respectively. These

expression vectors have the DNA (nucleotide) sequences SEQ ID NO 15, SEQ ID NO 16, SEQ ID NO 17, or SEQ ID NO 18, respectively.

Construction of humanised antibody heavy and light chain expression vectors

5 Human kappa light chain expression vectors for versions VLh and VLm

In order to construct the final expression vector encoding for the complete humanised light chain of human kappa isotype, DNA fragments encoding the complete light chain variable regions (VLh and VLm) were excised from the VLh and VLm containing PCR-Script cloning vectors (Stratagene) (VLm region) using HindIII and BglII. The gel-purified fragments were
10 then subcloned into the HindIII and BamHI sites of C21-HCMV Kappa expression vector which was created during construction of the humanised anti-IgE antibody TESC-21 (Kolbinger et al 1993) and which originally received from M. Bendig (MRC Collaborative Centre, London, UK) (Maeda et al. 1991). The ligation products were purified by phenol/chloroform extraction, and electroporated into electrocoporation-competent Epicurian
15 Coli® XL1-Blue strain (Cat. N° #200228, Stratagene). After plating on LB/amp agar plates overnight at 37°C, each 12 colonies were picked to prepare plasmid DNA from a 3 ml culture using the BioRobot 9600 (Qiagen). This yielded the light chain expression vectors for the humanised antibody versions VLh and VLm, respectively, as further described in the Figures.

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Human gamma-1 heavy chain expression vectors for VHQ

For the construction of the VHQ expression vector, a step-wise approach was taken. First, the complete variable region of VHQ was assembled by PCR according to the methodology as described in Kolbinger et al 1993 (Protein Eng. 1993 Nov; 6(8):971-80) and subcloned into
25 the C21-HCMV-gamma-1 expression from which the C21 insert had been removed using the same enzymes. A HindIII/BamHI fragment of PCRScript clone VHQ containing the complete variable region was then subcloned into expression vector C21-HCMV-gamma-1 cleaved with the same enzymes. This yielded the final expression vector for the humanised antibody version VHQ.

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Human gamma-1 heavy chain expression vectors for VHE

The construction of the final VHE expression vector encoding for the complete humanised heavy chain of human gamma-1 isotype was achieved by directly ligating a HindIII and BamHI restricted PCR fragment encoding the variable region into the HindIII and BamHI sites of C21-HCMV gamma-1 expression vector which was created during construction of the humanised anti-IgE antibody TESC-21 (Kolbinger et al 1993) and which was also originally received from M. Bendig (MRC Collaborative Centre, London, UK) (Maeda et al. 1991).

10 Transient expression in COS cells

The following transfection protocol is adapted for adherent COS cells in 150 mm cell culture dishes, using SuperFect™ Transfection Reagent (Cat. N°301305, Qiagen). The four different expression vectors described above are used for transient transfection of cells. For expression of humanised antibody, each of two clones containing heavy chain inserts (VHE or VHQ, respectively) are co-transfected into cells with each of the two clones encoding for the light chains (humV1 or humV2, respectively), in total 4 different combinations of heavy and light chain expression vectors (VHE/humV1, VHE/humV2, VHQ/humV1 and VHQ/humV2). Before transfection, the plasmids are linearized with the restriction

endonuclease PvuI which cleaves in the region encoding the resistance gene for ampicillin. The day before transfection, 4×10^6 COS cells in 30 ml of fresh culture medium are seeded in 150 mm cell culture dishes. Seeding at this cell density generally yielded 80% confluency after 24 hours. On the day of transfection, four different combinations of linearized heavy- and light-chain DNA expression vectors (15 µg each) are diluted in a total volume of 900 µl of fresh medium without serum and antibiotics. 180 µl of SuperFect Transfection Reagent is then mixed thoroughly with the DNA solution. The DNA mixture is incubated for 10 min at room temperature to allow complex formation. While complex formation takes place, the growth medium is removed from COS cell cultures, and cells are washed once with PBS. 9 ml of fresh culture medium (containing 10% FBS and antibiotics) are then added to each reaction tube containing the transfection complexes and well mixed. The final preparation is immediately transferred to each of 4 cultures to be transfected and gently mixed. Cell cultures are then incubated with the DNA complexes for 3 hours at 37°C and 5% CO₂. After incubation, the medium containing transfection complexes is removed and replaced with 30

ml of fresh culture medium. At 48 hr post transfection, the culture supernatants are harvested.

Concentration of culture supernatants

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For ELISA and FACS analysis, the culture supernatants collected from COS cells transfected with heavy- and light- chain plasmids are concentrated as follows. 10 ml of each supernatant are added to Centriprep YM-50 Centrifugal Filter Devices (Cat. N° 4310, Millipore) as described by the manufacturer. The Centriprep filters are centrifuged for 10 min at 3000 rpm at room temperature. The centrifugation step is then repeated again with the remaining 20 ml of supernatant using only 5 min of centrifugation and supervising the concentration evolution. The intermediate 500 µl of concentrated supernatant is recovered, transferred to new Microcon Centrifugal Filter Devices (Cat. N° 42412, Microcon) and further concentrated following the manufacturer's protocol. The concentrated supernatants are centrifuged four times for 24 min at 3000 rpm at room temperature, one time for 10 min at 6000 rpm and then, three times for 5 min, always supervising the concentration evolution. The final volume of concentrated conditioned medium achieved is 100-120 µl corresponding to a 250 to 300-fold concentration of original culture medium and is stored at 4°C until use. For comparison and control, culture medium from untransfected cells is similarly concentrated, using the same centrifugation protocol described above.

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Generation of stable Sp2/0 myeloma transfectants secreting humanised anti-CD45RO/RB antibodies

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The mouse myeloma cell line Sp2/0 (ATCC, CRL-1581) is electroporated with vectors encoding heavy (VHE or VHQ) and light (humV1 or humV2) chain of the CD45RO/RB binding humanised antibodies. Four different combinations of heavy and light chain expression vectors (VHE/humV1, VHE/humV2; VHQ/humV1 and VHQ/humV2) are transfected according to the following protocol: 20 µg supercoiled DNA of each plasmid is mixed in an electroporation cuvette (0.4 cm gap) with 8×10^6 live Sp2/0 cells suspended in DMEM / 10%FCS culture medium. Electroporation settings are 1500 V, 25 µF using a BioRad GenePulser instrument. After electroporation, cells are cultured for 20 h in culture medium (DMEM supplemented with 10% FCS penicillin, streptomycin and L-glutamine). On day two the selection drug G418 (Cat. N° 10131-019, Gibco) is added to a final

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concentration of 1 mg active drug / ml and the cells are distributed into one 96-well plate, 200 µl each well with approx. 10^5 cells per well. Ten to 15 days later, G418-surviving clones are expanded in G418-containing medium. Secretion of humanised mAbs from these transfectants is assessed by ELISA, using a coating antibody goat anti-human IgG/Fcγ (Cat. N° 109-005-098, Jackson Labs) and a peroxidase-coupled antibody against human kappa light chain (Cat. N° A-7164, Sigma). Transfectants, which score positive in this assay are selected for a comparison of productivity on a per cell per day basis, again using ELISA (see below). The best clone of each transfectant is selected for immediate subcloning by limiting dilution, using a seeding density of 1 cell per well. Productivity of G418-surviving subclones is again determined as described above. Subclones are expanded in G418-containing selection medium, until the culture volume reaches 150 ml, at which stage the culture is continued without G418 in flasks destined to feed roller bottles.

After the first transfection and selection, stable transfectants grow out of the 96-well plates at a frequency of 20.8 % for VHE/humV1, 11.5 % for VHQ/humV1, 18.8 % for VHE/humV2 and 7.3 % for VHQ/humV2. After two rounds of subcloning the best two producers are clone 1.33.25 (3.87 pg/cell/day) and clone 1.33.26 (3.43 pg/cell/day) for VHE/humV1 and clone 12.1.4 (1.19 pg/cell/day) and clone 12.1.20 (1.05 pg/cell/day) for VHQ/humV1. The stable Sp2/0 transfectants for VHE/humV1 and VHQ/humV1 are subsequently expanded for antibody production and purification.

The antibodies are purified from supernatants of stably transfected SP2/0 myeloma cell lines containing 10% FCS by a combination of affinity chromatography using an immobilized anti-human IgG/Fc matrix and size-exclusion chromatography. If required, endotoxin is removed using an Acticlean Etox column (Sterogene Bioseparations).

Example 5: Determination of recombinant human IgG expression by ELISA

To determine IgG concentrations of recombinant human antibody expressed in the culture supernatants, a sandwich ELISA protocol has been developed and optimized using human IgG as standard. Flat bottom 96-well microtiter plates (Cat. N° 4-39454, Nunc Immunoplate Maxisorp) are coated overnight at 4°C with 100 µl of goat anti-human IgG (whole molecule, Cat. N° I1011, SIGMA) at the final concentration of 0.5 µg/ml in PBS. Wells are then washed 3 times with washing buffer (PBS containing 0.05% Tween 20) and blocked for 1.5 hours at

37°C with blocking buffer (0.5% BSA in PBS). After 3 washing cycles, the antibody samples and the standard human IgG (Cat.No. I4506, SIGMA) are prepared by serial 1.5-fold dilution in blocking buffer. 100 µl of diluted samples or standard are transferred in duplicate to the coated plate and incubated for 1 hour at room temperature. After incubation, the plates are washed 3 times with washing buffer and subsequently incubated for 1 hour with 100 µl of horseradish peroxidase-conjugated goat anti-human IgG kappa-light chain (Cat. N° A-7164, SIGMA) diluted at 1/4000 in blocking buffer. Control wells received 100 µl of blocking buffer or concentrated normal culture medium. After washing, the colorimetric quantification of bound peroxidase in the sample and standard wells is performed, using a TMB Peroxidase EIA Substrate Kit (Cat. N° 172-1067, Bio-Rad) according to the manufacturer's instructions. The peroxidase mixture is added at 100 µl per well and incubated for 30 min at room temperature in the dark. The colorimetric reaction is stopped by addition of 100 µl of 1 M sulfuric acid and the absorbance in each well is read at 450 nm, using an ELISA plate reader (Model 3350-UV, BioRad).

With a correlation coefficient of 0.998 for the IgG standard curve, the following concentrations are determined for the four different culture concentrates (ca. 250-300 fold concentrated) obtained from transfected COS cells:

VHE/humV1 supernatant = 8.26 µg/ml
VHE/humV2 supernatant = 6.27 µg/ml
VHQ/humV1 supernatant = 5.3 µg/ml
VHQ/humV2 supernatant = 5.56 µg/ml

Example 6: FACS competition analysis (binding affinity)

The human T-cell line PEER is chosen as the target cell for FACS analysis because it expressed the CD45 antigen on its cell surface. To analyze the binding affinity of humanised antibody supernatants, competition experiments using FITC-labeled chimeric antibody as a reference are performed and compared with the inhibition of purified mouse antibody and of chimeric antibody. PEER cell cultures are centrifuged for 10 seconds at 3000 rpm and the medium is removed. Cells are resuspended in FACS buffer (PBS containing 1% FBS and 0.1% sodium azide) and seeded into 96-well round-bottom microtiter plate at a cell density of 1×10^5 cells per well. The plate is centrifuged and the supernatant is discarded. For

blocking studies, 25 µl of concentrated untransfected medium or isotype matched control antibody (negative controls), unlabeled mouse antibody or chimeric antibody (positive controls) as well as concentrated supernatant containing the various combinations of humanised antibody (samples), is first added in each well at the indicated concentrations in the text. After 1 hour of incubation at 4°C, PEER cells are washed with 200 µl of FACS buffer by centrifugation. Cells are subsequently incubated for 1 hour at 4°C with chimeric antibody conjugated with FITC in 25 µl of FACS buffer at the final concentration of 20 µg/ml. Cells are washed and resuspended in 300 µl of FACS buffer containing 2 µg/ml propidium iodide which allows gating of viable cells. The cell preparations are analyzed on a flow cytometer (FACSCalibur, Becton Dickinson).

FACS analyses indicate a dose-dependent blockade of fluorochrome-labeled chimeric antibody by the concentrated humanised antibody culture supernatants. No dose-dependent blockade of chimeric antibody binding is seen with the isotype matched control antibody, indicating that the blocking effect by the different humanised antibody combinations is epitope specific and that epitope specificity appears to be retained after the humanisation process.

Undiluted supernatant from the above mentioned SP2/0 transfectants or chimeric antibody (positive controls) or isotype matched control antibody (negative controls) at 2 µg/ml in culture medium are incubated with 1.5×10^5 PEER cells in 100 µl for 30 min at 4°C. Then, 100 µl PBS containing FITC-labeled chimeric antibody is added to each sample and incubation at 4°C continues for another 30 minutes. After washing, cells are resuspended in FACS-PBS containing 1 µg/ml 7-Amino-Actinomycin D and analyzed by flow cytometry using a Becton Dickinson FACSCalibur instrument and the CellQuest Pro Software. Gating was on live cells, i.e. 7-Amino-Actinomycin D – negative events.

FACS analyses show that unlabeled humanised CD45RB/RO binding molecules, e.g. VHE/humV1 and VHQ/humV1 but not the isotype matched control antibody compete with FITC-labeled chimeric antibody for binding to the human CD45-positive T cell line PEER.

Example 7: Biological activities of CD45RB/RO binding molecules

In this study, we have addressed whether CD45RB/RO binding chimeric antibody, when present in cultures of polyclonally activated primary human T cells (i) supports the differentiation of T cells with a characteristic Treg phenotype, (ii) prevents or enhances apoptosis following T cell activation, and (iii) affects expression of subset-specific antigens and receptors after restimulation.

CD45RB/RO binding chimeric antibody enhances cell death in polyclonally activated T cells

Primary T cells (mixture of CD4⁺ and CD8⁺ T subsets) were subjected to activation by anti-CD3 plus anti-CD28 mAb (200 ng/ml each) in the presence or absence (=control) of CD45RB/RO binding chimeric antibody. Excess antibodies were removed by washing on day 2. 7-amino-actinomycin D (7-AAD) as a DNA-staining dye taken up by apoptotic and necrotic cells was used to measure cell death following activation. The results show that activation of T cells in the presence of CD45RB/RO binding chimeric antibody increased the fraction of 7-AAD positive cells than two-fold on day 2 after activation. On day 7, the portion of 7-AAD positive cells was again similar in CD45RB/RO binding chimeric antibody-treated and control cultures.

CD45RB/RO binding chimeric antibody but not control mAb treated T cells display a T regulatory cell (Treg) phenotype

Increased expression of CD25 and the negative regulatory protein CTLA-4 (CD152) is a marker of Treg cells. Functional suppression of primary and secondary T cell responses by CD45RB/RO binding chimeric antibody may be due to the induction of Treg cells. To address this issue, T cells were activated by anti-CD3 + CD28 mAbs and cultured in the presence of CD45RB/RO binding chimeric antibody or anti-LPS control mAb. The time course of CTLA-4 and CD25 expression reveals marked differences between controls and CD45RB/RO binding chimeric antibody-treated T cells on days 1 and 3 after secondary stimulation, indicating a Treg phenotype.

Intracellular CTLA-4 expression is sustained in the presence of CD45RB/RO binding chimeric antibody

It has been reported that substantial amounts of CTLA-4 can also be found intracellularly. Therefore, in parallel to surface CTLA-4 staining, intracellular CTLA-4 expression was analyzed. Moderate differences between T cell cultures were seen on day 4 after stimulation. After prolonged culture, however, high levels of intracellular CTLA-4 were sustained only in CD45RB/RO binding chimeric antibody-treated but not in control T cells.

CD45RB/RO binding chimeric antibody-treated T cells become double positive for CD4 and CD8

Following stimulation, T cells induce and upregulate the expression of several surface receptors, such as CD25, CD152 (CTLA-4), CD154 (CD40-Ligand) and others. In contrast, the level of expression of CD4 or CD8 is thought to stay relatively constant. We reproducibly observed a strong increase of both CD4 and CD8 antigens on CD45RB/RO binding chimeric antibody-treated but not on control Ab-treated T cells after activation. The emergence of a CD4/CD8 double-positive T cell population seems to be due to the upregulation of CD4 on the CD8+ subset and conversely, CD8 on the CD4+ subset. This contrasts with a moderately low percentage of double positive T cells in control cultures.

High IL-2 receptor alpha-chain, but very low beta-chain expression by CD45RB/RO binding chimeric antibody-treated T cells

Treg cells are known to be constitutively positive for CD25, the IL-2 receptor alpha-chain. The regulation of other subunits of the trimeric IL-2 receptor on Treg cells is not known. Recently we have compared the expression of the beta-chain of IL-2 receptor, e.g. CD122, on T cells activated and propagated in the presence or absence of CD45RB/RO binding chimeric antibody. The results show that CD45RB/RO binding chimeric antibody-treated T cells have about ten-fold lower CD122 expression as compared to T cells in control cultures. This difference may indicate that Treg cells require factors other than IL-2 to proliferate.

Example 8: Sequences of the invention (CDR sequences of the invention are underlined)

SEQ ID NO:1

5 Part of the amino acid sequence of chimeric light chain

DILLTQSPAILSVSPGERVSFSCRASQNIGTSIQWYQQRTNGSPRLLIRSSSESISGIPSRFSG
SGSGTDFTLSINSVESEDIADYYCQQSNTWPFTFGSGTKLEIK

SEQ ID NO:2

10 Part of the amino acid sequence of chimeric heavy chain

EVQLQQSGPELVKPGASVKMSCKASGYTFTNYIIHWVKQEPGQGLEWIGYFNPYNHGTY
NEKFKGRATLTADKSSNTAYMDLSSLTSEDSAIYYCARSGPYAWFDTWGQGTTVTVSS

SEQ ID NO:3

15 Amino acid sequence of chimeric light chain

DILLTQSPAILSVSPGERVSFSCRASQNIGTSIQWYQQRTNGSPRLLIRSSSESISGIPSRFSG
SGSGTDFTLSINSVESEDIADYYCQQSNTWPFTFGSGTKLEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC

20

SEQ ID NO:4

Amino acid sequence of chimeric heavy chain

EVQLQQSGPELVKPGASVKMSCKASGYTFTNYIIHWVKQEPGQGLEWIGYFNPYNHGTY
NEKFKGRATLTADKSSNTAYMDLSSLTSEDSAIYYCARSGPYAWFDTWGQGTTVTVSSAS

25

TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS

30

CSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:5**Nucleotide sequence encoding a polypeptide of SEQ ID NO:1**

GACATTCTGCTGACCCAGTCTCCAGCCATCCTGTCTGTGAGTCCAGGAGAAAGAGTCA
GTTTCTCCTGCAGGGCCAGTCAGAACATTGGCACAAGCATACAGTGGTATCAACAAAGA
5 ACAAATGGTTCTCCAAGGCTTCTCATAAGGTCTTCTTCTGAGTCTATCTCTGGGATCCCT
TCCAGGTTTAGTGGCAGTGGATCAGGGACAGATTTTACTCTTAGCATCAACAGTGTGGA
GTCTGAAGATATTGCAGATTATTACTGTCAACAAAGTAATACCTGGCCATTCACGTTTCGG
CTCGGGGACCAAGCTTGAAATCAAA

SEQ ID NO:6**Nucleotide sequence encoding a polypeptide of SEQ ID NO:2**

GAGGTGCAGCTGCAGCAGTCAGGACCTGAACTGGTAAAGCCTGGGGCTTCAGTGAAG
ATGTCCTGCAAGGCCTCTGGATACACATTCACTAATTATATTATCCACTGGGTGAAGCA
GGAGCCTGGTCAGGGCCTTGAATGGATTGGATATTTAATCCTTACAATCATGGTACTA
15 AGTACAATGAGAAGTTCAAAGGCAGGGCCACACTAACTGCAGACAAATCCTCCAACACA
GCCTACATGGACCTCAGCAGCCTGACCTCTGAGGACTCTGCGATCTACTACTGTGCAA
GATCAGGACCCTATGCCTGGTTTGACACCTGGGGCCAAGGGACCACGGTCACCGTCTC
CTCA

SEQ ID NO:7**Part of amino acid sequence of humanised light chain designated humV2 (humV2 = VLm)**

DILLTQSPAT LSLSPGERAT FSCRASQNIG TSIQWYQQKT NGAPRLLIRS SSESISGIPS
RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ SNTWPFTFGQ GTKLEIK

25

SEQ ID NO:8**Part of amino acid sequence of humanised light chain designated humV1 (humV1 = VLh)**

DILLTQSPAT LSLSPGERAT LSCRASQNIG TSIQWYQQKP GQAPRLLIRS SSESISGIPS
30 RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ SNTWPFTFGQ GTKLEIK

SEQ ID NO:9

Part of amino acid sequence of humanised heavy chain designated VHE
 EVQLVESGAE VKKPGASVKV SCKASGYTFT NYIIHWVKQE PGQGLEWIGY
FNPYNHGTKY NEKFKGRATL TANKSISTAY MELSSLRSED TAVYYCARSG
 5 PYAWFDTWGQ GTTVTVSS

SEQ ID NO:10

Part of amino acid sequence of humanised heavy chain designated VHQ
 QVQLVESGAE VKKPGASVKV SCKASGYTFT NYIIHWVKQE PGQGLEWIGY
 10 FNPYNHGTKY NEKFKGRATL TANKSISTAY MELSSLRSED TAVYYCARSG
PYAWFDTWGQ GTTVTVSS

SEQ ID NO:11

Nucleotide sequence encoding amino acid sequence SEQ ID NO:9

15 GAGGTGCAGCTGGTGGAGTCAGGAGCCGAAGTGAAAAAGCCTGGGGCTTCAGTGAAG
 GTGTCCTGCAAGGCCTCTGGATACACATTCACTAATTATATTATCCACTGGGTGAAGCA
 GGAGCCTGGTCAGGGCCTTGAATGGATTGGATATTTTAATCCTTACAATCATGGTACTA
 AGTACAATGAGAAGTTCAAAGGCAGGGCCACACTAACTGCAAACAAATCCATCAGCACA
 GCCTACATGGAGCTCAGCAGCCTGCGCTCTGAGGACACTGCGGTCTACTACTGTGCAA
 20 GATCAGGACCCTATGCCTGGTTTGACACCTGGGGCCAAGGGACCACGGTCACCGTCTC
 CTCA

SEQ ID NO:12

Nucleotide sequence encoding amino acid sequence SEQ ID NO:10

25 CAGGTGCAGCTGGTGGAGTCAGGAGCCGAAGTGAAAAAGCCTGGGGCTTCAGTGAAG
 GTGTCCTGCAAGGCCTCTGGATACACATTCACTAATTATATTATCCACTGGGTGAAGCA
 GGAGCCTGGTCAGGGCCTTGAATGGATTGGATATTTTAATCCTTACAATCATGGTACTA
 AGTACAATGAGAAGTTCAAAGGCAGGGCCACACTAACTGCAAACAAATCCATCAGCACA
 GCCTACATGGAGCTCAGCAGCCTGCGCTCTGAGGACACTGCGGTCTACTACTGTGCAA
 30 GATCAGGACCCTATGCCTGGTTTGACACCTGGGGCCAAGGGACCACGGTCACCGTCTC
 CTCA

SEQ ID NO:13**Nucleotide sequence encoding amino acid sequence SEQ ID NO:7**

GACATTCTGCTGACCCAGTCTCCAGCCACCCTGTCTCTGAGTCCAGGAGAAAGAGCCA
 CTTTCTCCTGCAGGGCCAGTCAGAACATTGGCACAAGCATACAGTGGTATCAACAAAAA
 5 ACAAATGGTGCTCCAAGGCTTCTCATAAGGTCTTCTTCTGAGTCTATCTCTGGGATCCC
 TTCCAGGTTTAGTGGCAGTGGATCAGGGACAGATTTTACTCTTACCATCAGCAGTCTGG
 AGCCTGAAGATTTTGCAGTGTATTACTGTCAACAAAGTAATACCTGGCCATTACAGTTC
 GGCCAGGGGACCAAGCTGGAGATCAAA

10 **SEQ ID NO:14****Nucleotide sequence encoding amino acid sequence SEQ ID NO:8**

GACATTCTGCTGACCCAGTCTCCAGCCACCCTGTCTCTGAGTCCAGGAGAAAGAGCCA
 CTCTCTCCTGCAGGGCCAGTCAGAACATTGGCACAAGCATACAGTGGTATCAACAAAAA
 CCAGGTCAGGCTCCAAGGCTTCTCATAAGGTCTTCTTCTGAGTCTATCTCTGGGATCCC
 15 TTCCAGGTTTAGTGGCAGTGGATCAGGGACAGATTTTACTCTTACCATCAGCAGTCTGG
 AGCCTGAAGATTTTGCAGTGTATTACTGTCAACAAAGTAATACCTGGCCATTACAGTTC
 GGCCAGGGGACCAAGCTGGAGATCAAA

SEQ ID NO:1520 **Nucleotide sequence of the expression vector HCMV-G1 HuAb-VHQ**

**(Complete DNA Sequence of a humanised heavy chain expression vector comprising
 SEQ ID NO:12 (VHQ) from 3921-4274)**

1 AGCTTTTTGC AAAAGCCTAG GCCTCCAAAA AAGCCTCCTC ACTACTTCTG
 25 51 GAATAGCTCA GAGGCCGAGG CGGCCTCGGC CTCTGCATAA ATAAAAAAAAA
 101 TTAGTCAGCC ATGGGGCGGA GAATGGGCGG AACTGGGCGG AGTTAGGGGC
 151 GGGATGGGCG GAGTTAGGGG CGGGACTATG GTTGCTGACT AATTGAGATG
 201 CATGCTTTGC ATACTTCTGC CTGCTGGGGA GCCTGGTTGC TGAATAATTG
 251 AGATGCATGC TTTGCATACT TCTGCCTGCT GGGGAGCCTG GGGACTTTCC
 30 301 ACACCCTAAC TGACACACAT TCCACAGCTG CCTCGCGCGT TTCGGTGATG
 351 ACGGTGAAAA CCTCTGACAC ATGCAGCTCC CGGAGACGGT CACAGCTTGT
 401 CTGTAAGCGG ATGCCGGGAG CAGACAAGCC CGTCAGGGCG CGTCAGCGGG
 451 TGTTGGCGGG TGTCGGGGCG CAGCCATGAC CCAGTCACGT AGCGATAGCG
 501 GAGTGTATAC TGGCTTAAC TATGCGGCATC AGAGCAGATT GTACTGAGAG
 35 551 TGCACCATAT GCGGTGTGAA ATACGCACA GATGCGTAAG GAGAAAATAC

	601	CGCATCAGGC	GCTCTTCCGC	TTCCTCGCTC	ACTGACTCGC	TGCGCTCGGT
	651	CGTTCGGCTG	CGGCGAGCGG	TATCAGCTCA	CTCAAAGGCG	GTAATACGGT
	701	TATCCACAGA	ATCAGGGGAT	AACGCAGGAA	AGAACATGTG	AGCAAAAGGC
	751	CAGCAAAAGG	CCAGGAACCG	TAAAAAGGCC	GCGTTGCTGG	CGTTTTTCCA
5	801	TAGGCTCCGC	CCCCCTGACG	AGCATCACAA	AAATCGACGC	TCAAGTCAGA
	851	GGTGGCGAAA	CCCGACAGGA	CTATAAAGAT	ACCAGGCGTT	TCCCCCTGGA
	901	AGCTCCCTCG	TGCGCTCTCC	TGTTCCGACC	CTGCCGCTTA	CCGGATACCT
	951	GTCCGCCTTT	CTCCCTTCGG	GAAGCGTGGC	GCTTTCTCAT	AGCTCACGCT
	1001	GTAGGTATCT	CAGTTCGGTG	TAGGTCGTTT	GCTCCAAGCT	GGGCTGTGTG
10	1051	CACGAACCCC	CCGTTTCAGCC	CGACCGCTGC	GCCTTATCCG	GTAAGTATCG
	1101	TCTTGAGTCC	AACCCGGTAA	GACACGACTT	ATCGCCACTG	GCAGCAGCCA
	1151	CTGGTAACAG	GATTAGCAGA	GCGAGGTATG	TAGGCGGTGC	TACAGAGTTC
	1201	TTGAAGTGGT	GGCCTAACTA	CGGCTACACT	AGAAGGACAG	TATTTGGTAT
	1251	CTGCGCTCTG	CTGAAGCCAG	TTACCTTCGG	AAAAAGAGTT	GGTAGCTCTT
15	1301	GATCCGGCAA	ACAAACCACC	GCTGGTAGCG	GTGGTTTTTT	TGTTTGCAAG
	1351	CAGCAGATTA	CGCGCAGAAA	AAAAGGATCT	CAAGAAGATC	CTTTGATCTT
	1401	TTCTACGGGG	TCTGACGCTC	AGTGGAACGA	AAACTCACGT	TAAGGGATTT
	1451	TGGTCATGAG	ATTATCAAAA	AGGATCTTCA	CCTAGATCCT	TTTAAATTAA
	1501	AAATGAAGTT	TTAAATCAAT	CTAAAGTATA	TATGAGTAAA	CTTGGTCTGA
20	1551	CAGTTACCAA	TGCTTAATCA	GTGAGGCACC	TATCTCAGCG	ATCTGTCTAT
	1601	TTCGTTTCATC	CATAGTTGCC	TGACTCCCCG	TCGTGTAGAT	AACTACGATA
	1651	CGGGAGGGCT	TACCATCTGG	CCCCAGTGCT	GCAATGATAC	CGCGAGACCC
	1701	ACGCTCACCG	GCTCCAGATT	TATCAGCAAT	AAACCAGCCA	GCCGGAAGGG
	1751	CCGAGCGCAG	AAGTGGTCCT	GCAACTTTAT	CCGCCTCCAT	CCAGTCTATT
25	1801	AATTGTTGCC	GGGAAGCTAG	AGTAAGTAGT	TCGCCAGTTA	ATAGTTTGCG
	1851	CAACGTTGTT	GCCATTGCTG	CAGGCATCGT	GGTGTACGCG	TCGTCGTTTG
	1901	GTATGGCTTC	ATTCAGCTCC	GGTTCCCAAC	GATCAAGGCG	AGTTACATGA
	1951	TCCCCCATGT	TGTGCAAAAA	AGCGGTTAGC	TCCTTCGGTC	CTCCGATCGT
	2001	TGTCAGAAGT	AAGTTGGCCG	CAGTGTTATC	ACTCATGGTT	ATGGCAGCAC
30	2051	TGCATAATTC	TCTTACTGTC	ATGCCATCCG	TAAGATGCTT	TTCTGTGACT
	2101	GGTGAGTACT	CAACCAAGTC	ATTCTGAGAA	TAGTGTATGC	GGCGACCGAG
	2151	TTGCTCTTGC	CCGGCGTCAA	CACGGGATAA	TACCGCGCCA	CATAGCAGAA
	2201	CTTTAAAAGT	GCTCATCATT	GGAAAACGTT	CTTCGGGGCG	AAAACCTCTCA
	2251	AGGATCTTAC	CGCTGTTGAG	ATCCAGTTTC	ATGTAACCCA	CTCGTGCACC
35	2301	CAACTGATCT	TCAGCATCTT	TTACTTTTAC	CAGCGTTTCT	GGGTGAGCAA
	2351	AAACAGGAAG	GCAAAATGCC	GCAAAAAAGG	GAATAAGGGC	GACACGAAAA
	2401	TGTTGAATAC	TCATACTCTT	CCTTTTTTCAA	TATTATTGAA	GCATTTATCA

2451 GGGTTATTGT CTCATGAGCG GATACATATT TGAATGTATT TAGAAAAATA
 2501 AACAAATAGG GGTTCCGCGC ACATTTCCCC GAAAAGTGCC ACCTGACGTC
 2551 TAAGAAACCA TTATTATCAT GACATTAACC TATAAAAATA GGCGTATCAC
 2601 GAGGCCCTTT CGTCTTCAAG AATTCAGCTT GGCTGCAGTG AATAATAAAA
 5 2651 TGTGTGTTTG TCCGAAATAC GCGTTTTGAG ATTTCTGTCTG CCGACTAAAT
 2701 TCATGTCGCG CGATAGTGGT GTTTATCGCC GATAGAGATG GCGATATTGG
 2751 AAAAATCGAT ATTTGAAAAT ATGGCATATT GAAAATGTCTG CCGATGTGAG
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 2851 CGCGATATCT GGCGATAGCG CTTATATCGT TTACGGGGGA TGGCGATAGA
 10 2901 CGACTTTGGT GACTTGGGCG ATTCTGTGTG TCGCAAATAT CGCAGTTTCG
 2951 ATATAGGTGA CAGACGATAT GAGGCTATAT CGCCGATAGA GGCGACATCA
 3001 AGCTGGCACA TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG
 3051 CCATTAGCCA TATTATTCAT TGGTTATATA GCATAAATCA ATATTGGCTA
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 15 3151 GCTCATGTCC AACATTACCG CCATGTTGAC ATTGATTATT GACTAGTTAT
 3201 TAATAGTAAT CAATTACGGG GTCATTAGTT CATAGCCCAT ATATGGAGTT
 3251 CCGCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA CCGCCCAACG
 3301 ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA
 3351 ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTTAC GGTAAACTGC
 20 3401 CCACTTGGA GTACATCAAG TGTATCATAT GCCAAGTACG CCCCCTATTG
 3451 ACGTCAATGA CGGTAAATGG CCCGCCTGGC ATTATGCCCA GTACATGACC
 3501 TTATGGGACT TTCCTACTTG GCAGTACATC TACGTATTAG TCATCGCTAT
 3551 TACCATGGTG ATGCGGTTTT GGCAGTACAT CAATGGGCGT GGATAGCGGT
 3601 TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT
 25 3651 TTGTTTTGGC ACCAAAATCA ACGGGACTTT CCAAATGTC GTAACAACTC
 3701 CGCCCCATTG ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA
 3751 TAAGCAGAGC TCGTTTAGTG AACCGTCAGA TCGCCTGGAG ACGCCATCCA
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 30 3901 TGGCCCCCGG CGCCCACAGC CAGGTGCAGC TGGTGGAGTC AGGAGCCGAA
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 4051 GCCTTGAATG GATTGGATAT TTTAATCCTT ACAATCATGG TACTAAGTAC
 4101 AATGAGAAGT TCAAAGGCAG GGCCACACTA ACTGCAAACA AATCCATCAG
 35 4151 CACAGCCTAC ATGGAGCTCA GCAGCCTGCG CTCTGAGGAC ACTGCGGTCT
 4201 ACTACTGTGC AAGATCAGGA CCCTATGCCT GGTGTTGACAC CTGGGGCCAA
 4251 GGGACCACGG TCACCGTCTC CTCAGGTGAG TTCTAGAAGG ATCCCAAGCT

	4301	AGCTTTCTGG	GGCAGGCCAG	GCCTGACCTT	GGCTTTGGGG	CAGGGAGGGG
	4351	GCTAAGGTGA	GGCAGGTGGC	GCCAGCCAGG	TGCACACCCA	ATGCCCATGA
	4401	GCCCAGACAC	TGGACGCTGA	ACCTCGCGGA	CAGTTAAGAA	CCCAGGGGCC
	4451	TCTGCGCCCT	GGGCCCAGCT	CTGTCCCACA	CCGCGGTCAC	ATGGCACCAC
5	4501	CTCTCTTGCA	GCCTCCACCA	AGGGCCCATC	GGTCTTCCCC	CTGGCACCCCT
	4551	CCTCCAAGAG	CACCTCTGGG	GGCACAGCGG	CCCTGGGCTG	CCTGGTCAAG
	4601	GACTACTTCC	CCGAACCGGT	GACGGTGTCT	TGGAACTCAG	GCGCCCTGAC
	4651	CAGCGGCGTG	CACACCTTCC	CGGCTGTCTT	ACAGTCCTCA	GGACTCTACT
	4701	CCCTCAGCAG	CGTGGTGACC	GTGCCCTCCA	GCAGCTTGGG	CACCCAGACC
10	4751	TACATCTGCA	ACGTGAATCA	CAAGCCCAGC	AACACCAAGG	TGGACAAGAA
	4801	AGTTGGTGAG	AGGCCAGCAC	AGGGAGGGAG	GGTGTCTGCT	GGAAGCCAGG
	4851	CTCAGCGCTC	CTGCCTGGAC	GCATCCCGGC	TATGCAGCCC	CAGTCCAGGG
	4901	CAGCAAGGCA	GGCCCCGTCT	GCCTCTTCAC	CCGGAGGCCT	CTGCCCCGCC
	4951	CACTCATGCT	CAGGGAGAGG	GTCTTCTGGC	TTTTTCCCCA	GGCTCTGGGC
15	5001	AGGCACAGGC	TAGGTGCCCC	TAACCCAGGC	CCTGCACACA	AAGGGGCAGG
	5051	TGCTGGGCTC	AGACCTGCCA	AGAGCCATAT	CCGGGAGGAC	CCTGCCCCCTG
	5101	ACCTAAGCCC	ACCCCAAAGG	CCAAACTCTC	CACTCCCTCA	GCTCGGACAC
	5151	CTTCTCTCCT	CCCAGATTCC	AGTAACTCCC	AATCTTCTCT	CTGCAGAGCC
	5201	CAAATCTTGT	GACAAAATC	ACACATGCCC	ACCGTGCCCA	GGTAAGCCAG
20	5251	CCCAGGCCTC	GCCCTCCAGC	TCAAGGCGGG	ACAGGTGCCC	TAGAGTAGCC
	5301	TGCATCCAGG	GACAGGCCCC	AGCCGGGTGC	TGACACGTCC	ACCTCCATCT
	5351	CTTCCTCAGC	ACCTGAACTC	CTGGGGGGAC	CGTCAGTCTT	CCTCTTCCCC
	5401	CCAAAACCCA	AGGACACCCT	CATGATCTCC	CGGACCCCTG	AGGTCACATG
	5451	CGTGGTGCTG	GACGTGAGCC	ACGAAGACCC	TGAGGTCAAG	TTCAACTGGT
25	5501	ACGTGGACGG	CGTGGAGGTG	CATAATGCCA	AGACAAAGCC	GCGGGAGGAG
	5551	CAGTACAACA	GCACGTACCG	TGTGGTCAGC	GTCCTCACCG	TCCTGCACCA
	5601	GGACTGGCTG	AATGGCAAGG	AGTACAAGTG	CAAGGTCTCC	AACAAAGCCC
	5651	TCCCAGCCCC	CATCGAGAAA	ACCATCTCCA	AAGCCAAAGG	TGGGACCCGT
	5701	GGGGTGCGAG	GGCCACATGG	ACAGAGGCCG	GCTCGGCCCC	CCCTCTGCCC
30	5751	TGAGAGTGAC	CGCTGTACCA	ACCTCTGTCC	CTACAGGGCA	GCCCCGAGAA
	5801	CCACAGGTGT	ACACCCTGCC	CCCATCCCGG	GATGAGCTGA	CCAAGAACCA
	5851	GGTCAGCCTG	ACCTGCCTGG	TCAAAGGCTT	CTATCCCAGC	GACATCGCCG
	5901	TGGAGTGGGA	GAGCAATGGG	CAGCCGGAGA	ACAACCTACAA	GACCACGCCCT
	5951	CCCGTGCTGG	ACTCCGACGG	CTCCTTCTTC	CTCTACAGCA	AGCTCACCGT
35	6001	GGACAAGAGC	AGGTGGCAGC	AGGGGAACGT	CTTCTCATGC	TCCGTGATGC
	6051	ATGAGGCTCT	GCACAACCAC	TACACGCAGA	AGAGCCTCTC	CCTGTCTCCG
	6101	GGTAAATGAG	TGCGACGGCC	GGCAAGCCCC	CGCTCCCCCG	GCTCTCGCGG

6151 TCGCACGAGG ATGCTTGGCA CGTACCCCCT GTACATACTT CCCGGGCGCC
 6201 CAGCATGGAA ATAAAGCACC CAGCGCTGCC CTGGGCCCCT GCGAGACTGT
 6251 GATGGTTCTT TCCACGGGTC AGGCCGAGTC TGAGGCCTGA GTGGCATGAG
 6301 ATCTGATATC ATCGATGAAT TCGAGCTCGG TACCCGGGGA TCGATCCAGA
 5 6351 CATGATAAGA TACATTGATG AGTTTGGACA AACCACAAC TAGAATGCAGT
 6401 GAAAAAATG CTTTATTTGT GAAATTTGTG ATGCTATTGC TTTATTTGTA
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 10 6601 ACATCAAATA TTCCTTATTA ACCCCTTTAC AAATTAAAAA GCTAAAGGTA
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 6751 AGGTTACAGA ATATTTTTCC ATAATTTTCT TGTATAGCAG TGCAGCTTTT
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 15 6851 AGCATGACTC AAAAACTTA GCAATTCTGA AGGAAAGTCC TTGGGGTCTT
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 6951 GCCTCATCAT CACTAGATGG CATTCTTCT GAGCAAAACA GGTTTTCTC
 7001 ATTAAGGCA TTCCACCACT GCTCCCATTC ATCAGTTCCA TAGGTTGGAA
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 20 7101 AAAAATTTTA TATTTACCTT AGAGCTTTAA ATCTCTGTAG GTAGTTTGTC
 7151 CAATTATGTC ACACCACAGA AGTAAGGTTT CTTCAACAAG ATCCGGGACC
 7201 AAAGCGGCCA TCGTGCCTCC CCACTCCTGC AGTTCGGGGG CATGGATGCG
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 25 7351 AGGGGATCGA GCGCGGGGTG GGCGAAGAAC TCCAGCATGA GATCCCCGCG
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 7451 TTTCATAGAA GGCGGCGGTG GAATCGAAAT CTCGTGATGG CAGGTTGGGC
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 7551 TCAAGAAGGC GATAGAAGGC GATGCGCTGC GAATCGGGAG CGGCGATACC
 30 7601 GTAAAGCACG AGGAAGCGGT CAGCCCATTC GCCGCCAAGC TCTTCAGCAA
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 7701 CGGCCACAGT CGATGAATCC AGAAAAGCGG CCATTTTCCA CCATGATATT
 7751 CGGCAAGCAG GCATCGCCAT GGGTCACGAC GAGATCCTCG CCGTCGGGCA
 7801 TGCGCGCCTT GAGCCTGGCG AACAGTTCGG CTGGCGCGAG CCCCTGATGC
 35 7851 TCTTCGTCCA GATCATCCTG ATCGACAAGA CCGGCTTCCA TCCGAGTACG
 7901 TGCTCGCTCG ATGCGATGTT TCGCTTGGTG GTCGAATGGG CAGGTAGCCG
 7951 GATCAAGCGT ATGCAGCCGC CGCATTGCAT CAGCCATGAT GGATACTTTC

8001 TCGGCAGGAG CAAGGTGAGA TGACAGGAGA TCCTGCCCCG GCACTTCGCC
 8051 CAATAGCAGC CAGTCCCTTC CCGCTTCAGT GACAACGTCG AGCACAGCTG
 8101 CGCAAGGAAC GCCCGTCGTG GCCAGCCACG ATAGCCGCGC TGCCTCGTCC
 8151 TGCAGTTCAT TCAGGGCACC GGACAGGTCG GTCTTGACAA AAAGAACCGG
 5 8201 GCGCCCCCTGC GCTGACAGCC GGAACACGGC GGCATCAGAG CAGCCGATTG
 8251 TCTGTTGTGC CCAGTCATAG CCGAATAGCC TCTCCACCCA AGCGGCCCGA
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 8351 TGTCTCTTGA TCAGATCTTG ATCCCCTGCG CCATCAGATC CTTGGCGGCA
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 10 8451 GCCCCAGCTG GCAATTCCGG TTCGCTTGCT GTCCATAAAA CCGCCCAGTC
 8501 TAGCTATCGC CATGTAAGCC CACTGCAAGC TACCTGCTTT CTCTTTGCGC
 8551 TTGCGTTTTT CCTTGTCCAG ATAGCCCAGT AGCTGACATT CATCCGGGGT
 8601 CAGCACCGTT TCTGCGGACT GGCTTTCTAC GTGTTCCGCT TCCTTTAGCA
 8651 GCCCTTGCGC CCTGAGTGCT TCGGCAGCG TGAAGCT

15

SEQ ID NO:16**Nucleotide sequence of the expression vector HCMV-G1 HuAb-VHE****(Complete DNA Sequence of a humanised heavy chain expression vector comprising****SEQ ID NO: 11 (VHE) from 3921-4274)**

20

1 AGCTTTTTTGC AAAAGCCTAG GCCTCCAAAA AAGCCTCCTC ACTACTTCTG
 51 GAATAGCTCA GAGGCCGAGG CGGCCTCGGC CTCTGCATAA ATAAAAAAAAA
 101 TTAGTCAGCC ATGGGGCGGA GAATGGGCGG AACTGGGCGG AGTTAGGGGGC
 151 GGGATGGGCG GAGTTAGGGG CGGGACTATG GTTGCTGACT AATTGAGATG
 25 201 CATGCTTTGC ATACTTCTGC CTGCTGGGGA GCCTGGTTGC TGAATAATTG
 251 AGATGCATGC TTTGCATACT TCTGCCTGCT GGGGAGCCTG GGGACTTTCC
 301 ACACCCTAAC TGACACACAT TCCACAGCTG CCTCGCGCGT TTCGGTGATG
 351 ACGGTGAAAA CCTCTGACAC ATGCAGCTCC CGGAGACGGT CACAGCTTGT
 401 CTGTAAGCGG ATGCCGGGAG CAGACAAGCC CGTCAGGGCG CGTCAGCGGG
 30 451 TGTTGGCGGG TGTCGGGGCG CAGCCATGAC CCAGTCACGT AGCGATAGCG
 501 GAGTGTATAC TGGCTTAACT ATGCGGCATC AGAGCAGATT GTACTGAGAG
 551 TGCACCATAT GCGGTGTGAA ATACCGCACA GATGCGTAAG GAGAAAATAC
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 35 701 TATCCACAGA ATCAGGGGAT AACGCAGGAA AGAACATGTG AGCAAAAGGC
 751 CAGCAAAAGG CCAGGAACCG TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA

801 TAGGCTCCGC CCCCTGACG AGCATCACAA AAATCGACGC TCAAGTCAGA
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 5 1001 GTAGGTATCT CAGTTCGGTG TAGGTCGTTT GCTCCAAGCT GGGCTGTGTG
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 20 1751 CCGAGCGCAG AAGTGGTCCT GCAACTTTAT CCGCCTCCAT CCAGTCTATT
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 25 2001 TGTCAGAAAGT AAGTTGGCCG CAGTGTTATC ACTCATGGTT ATGGCAGCAC
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 30 2251 AGGATCTTAC CGCTGTTGAG ATCCAGTTCG ATGTAACCCA CTCGTGCACC
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 35 2501 AACAAATAGG GGTTCCGCGC ACATTTCCCC GAAAAGTGCC ACCTGACGTC
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 5 2851 CGCGATATCT GGCGATAGCG CTTATATCGT TTACGGGGGA TGGCGATAGA
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 25 3851 GCTTGCCGCC ACCATGGACT GGACCTGGAG GGTGTTCTGC CTGCTGGCCG
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	4501	CTCTCTTGCA	GCCTCCACCA	AGGGCCCATC	GGTCTTCCCC	CTGGCACCCCT
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	4651	CAGCGGCGTG	CACACCTTCC	CGGCTGTCCT	ACAGTCCTCA	GGACTCTACT
5	4701	CCCTCAGCAG	CGTGGTGACC	GTGCCCTCCA	GCAGCTTGGG	CACCCAGACC
	4751	TACATCTGCA	ACGTGAATCA	CAAGCCCAGC	AACACCAAGG	TGGACAAGAA
	4801	AGTTGGTGAG	AGGCCAGCAC	AGGGAGGGAG	GGTGTCTGCT	GGAAGCCAGG
	4851	CTCAGCGCTC	CTGCCTGGAC	GCATCCCGGC	TATGCAGCCC	CAGTCCAGGG
	4901	CAGCAAGGCA	GGCCCCGTCT	GCCTCTTCAC	CCGAGAGCCT	CTGCCCCGCC
10	4951	CACTCATGCT	CAGGGAGAGG	GTCTTCTGGC	TTTTTCCCCA	GGCTCTGGGC
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	5051	TGCTGGGCTC	AGACCTGCCA	AGAGCCATAT	CCGGGAGGAC	CCTGCCCCCTG
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	5151	CTTCTCTCCT	CCCAGATTCC	AGTAACTCCC	AATCTTCTCT	CTGCAGAGCC
15	5201	CAAATCTTGT	GACAAAACCTC	ACACATGCCC	ACCGTGCCCCA	GGTAAGCCAG
	5251	CCCAGGCCTC	GCCCTCCAGC	TCAAGGCGGG	ACAGGTGCCC	TAGAGTAGCC
	5301	TGCATCCAGG	GACAGGCCCC	AGCCGGGTGC	TGACACGTCC	ACCTCCATCT
	5351	CTTCCTCAGC	ACCTGAACTC	CTGGGGGGGAC	CGTCAGTCTT	CCTCTTCCCC
	5401	CCAAAACCCA	AGGACACCCT	CATGATCTCC	CGGACCCCTG	AGGTACACATG
20	5451	CGTGGTGGTG	GACGTGAGCC	ACGAAGACCC	TGAGGTCAAG	TTCAACTGGT
	5501	ACGTGGACGG	CGTGGAGGTG	CATAATGCCA	AGACAAAGCC	GCGGGAGGAG
	5551	CAGTACAACA	GCACGTACCG	TGTGGTCAGC	GTCCTCACCG	TCCTGCACCA
	5601	GGACTGGCTG	AATGGCAAGG	AGTACAAGTG	CAAGGTCTCC	AACAAAGCCC
	5651	TCCCAGCCCC	CATCGAGAAA	ACCATCTCCA	AAGCCAAAGG	TGGGACCCGT
25	5701	GGGGTGCGAG	GGCCACATGG	ACAGAGGCCG	GCTCGGCCCA	CCCTCTGCCC
	5751	TGAGAGTGAC	CGCTGTACCA	ACCTCTGTCC	CTACAGGGCA	GCCCCGAGAA
	5801	CCACAGGTGT	ACACCCTGCC	CCCATCCCGG	GATGAGCTGA	CCAAGAACCA
	5851	GGTCAGCCTG	ACCTGCCTGG	TCAAAGGCTT	CTATCCCAGC	GACATCGCCG
	5901	TGGAGTGGGA	GAGCAATGGG	CAGCCGGAGA	ACAACTACAA	GACCACGCCCT
30	5951	CCCGTGCTGG	ACTCCGACGG	CTCCTTCTTC	CTCTACAGCA	AGCTCACCGT
	6001	GGACAAGAGC	AGGTGGCAGC	AGGGGAACGT	CTTCTCATGC	TCCGTGATGC
	6051	ATGAGGCTCT	GCACAACCAC	TACACGCAGA	AGAGCCTCTC	CCTGTCTCCG
	6101	GGTAAATGAG	TGCGACGGCC	GGCAAGCCCC	CGCTCCCCGG	GCTCTCGCGG
	6151	TCGCACGAGG	ATGCTTGGCA	CGTACCCCTT	GTACATACTT	CCGGGGCGCC
35	6201	CAGCATGGAA	ATAAAGCACC	CAGCGCTGCC	CTGGGCCCCCT	GCGAGACTGT
	6251	GATGGTTCTT	TCCACGGGTC	AGGCCGAGTC	TGAGGCCTGA	GTGGCATGAG
	6301	ATCTGATATC	ATCGATGAAT	TCGAGCTCGG	TACCCGGGGA	TCGATCCAGA

	6351	CATGATAAGA	TACATTGATG	AGTTTGGACA	AACCACAACT	AGAATGCAGT
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	6451	ACCATTATAA	GCTGCAATAA	ACAAGTTAAC	AACAACAATT	GCATTCATTT
	6501	TATGTTTCAG	GTTTCAGGGG	AGGTGTGGGA	GGTTTTTTAA	AGCAAGTAAA
5	6551	ACCTCTACAA	ATGTGGTATG	GCTGATTATG	ATCTCTAGTC	AAGGCACTAT
	6601	ACATCAAATA	TTCCTTATTA	ACCCCTTTAC	AAATTAAGGTA	GCTAAAGGTA
	6651	CACAATTTTT	GAGCATAGTT	ATTAATAGCA	GACACTCTAT	GCCTGTGTGG
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	6751	AGGTTACAGA	ATATTTTTC	ATAATTTTCT	TGTATAGCAG	TGCAGCTTTT
10	6801	TCCTTTGTGG	TGTAAATAGC	AAAGCAAGCA	AGAGTTCTAT	TACTAAACAC
	6851	AGCATGACTC	AAAAAACTTA	GCAATTCTGA	AGGAAAGTCC	TTGGGGTCTT
	6901	CTACCTTTCT	CTTCTTTTTT	GGAGGAGTAG	AATGTTGAGA	GTCAGCAGTA
	6951	GCCTCATCAT	CACTAGATGG	CATTTCTTCT	GAGCAAAACA	GGTTTTCTCT
	7001	ATTAAAGGCA	TTCCACCACT	GCTCCCATTC	ATCAGTTCCA	TAGGTTGGAA
15	7051	TCTAAAATAC	ACAAACAATT	AGAATCAGTA	GTTTAACACA	TTATACACTT
	7101	AAAAATTTTA	TATTTACCTT	AGAGCTTTAA	ATCTCTGTAG	GTAGTTTGTG
	7151	CAATTATGTC	ACACCACAGA	AGTAAGGTTT	CTTCACAAAG	ATCCGGGACC
	7201	AAAGCGGCCA	TCGTGCCTCC	CCACTCCTGC	AGTTCGGGGG	CATGGATGCG
	7251	CGGATAGCCG	CTGCTGGTTT	CCTGGATGCC	GACGGATTTG	CACTGCCGGT
20	7301	AGAACTCCGC	GAGGTCGTCC	AGCCTCAGGC	AGCAGCTGAA	CCAACTCGCG
	7351	AGGGGATCGA	GCCCGGGGTG	GGCGAAGAAC	TCCAGCATGA	GATCCCCGCG
	7401	CTGGAGGATC	ATCCAGCCGG	CGTCCCGGAA	AACGATTCCG	AAGCCCAACC
	7451	TTTCATAGAA	GGCGGCGGTG	GAATCGAAAT	CTCGTGATGG	CAGGTTGGGC
	7501	GTCGCTTGGT	CGGTCATTTT	GAACCCCAAG	GTCCCGCTCA	GAAGAACTCG
25	7551	TCAAGAAGGC	GATAGAAGGC	GATGCGCTGC	GAATCGGGAG	CGGCGATACC
	7601	GTAAAGCACG	AGGAAGCGGT	CAGCCCATTC	GCCGCCAAGC	TCTTCAGCAA
	7651	TATCACGGGT	AGCCAACGCT	ATGTCCTGAT	AGCGGTCCGC	CACACCCAGC
	7701	CGGCCACAGT	CGATGAATCC	AGAAAAGCGG	CCATTTTCCA	CCATGATATT
	7751	CGGCAAGCAG	GCATCGCCAT	GGGTCACGAC	GAGATCCTCG	CCGTGGGGCA
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	7851	TCTTCGTCCA	GATCATCCTG	ATCGACAAGA	CCGGCTTCCA	TCCGAGTACG
	7901	TGCTCGCTCG	ATGCGATGTT	TCGCTTGGTG	GTCGAATGGG	CAGGTAGCCG
	7951	GATCAAGCGT	ATGCAGCCGC	CGCATTGCAT	CAGCCATGAT	GGATACTTTC
	8001	TCGGCAGGAG	CAAGGTGAGA	TGACAGGAGA	TCCTGCCCCG	GCACTTCGCC
35	8051	CAATAGCAGC	CAGTCCCTTC	CCGCTTCAGT	GACAACGTCG	AGCACAGCTG
	8101	CGCAAGGAAC	GCCCGTCGTG	GCCAGCCACG	ATAGCCGCGC	TGCCTCGTCC
	8151	TGCAGTTCAT	TCAGGGCACC	GGACAGGTCG	GTCTTGACAA	AAAGAACCGG

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8201 GCGCCCCTGC GCTGACAGCC GGAACACGGC GGCATCAGAG CAGCCGATTG
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8351 TGTCTCTTGA TCAGATCTTG ATCCCCTGCG CCATCAGATC CTTGGCGGCA
5 8401 AGAAAGCCAT CCAGTTTACT TTGCAGGGCT TCCCAACCTT ACCAGAGGGC
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8501 TAGCTATCGC CATGTAAGCC CACTGCAAGC TACCTGCTTT CTCTTTGCGC
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8601 CAGCACCGTT TCTGCGGACT GGCTTTCTAC GTGTTCCGCT TCCTTTAGCA
10 8651 GCCCTTGCGC CCTGAGTGCT TCGGCAGCG TGAAGCT

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SEQ ID NO:17**Nucleotide sequence of the expression vector HCMV-K HuAb-VL1 hum V1****(Complete DNA Sequence of a humanised light chain expression vector comprising****15 SEQ ID NO: 14 (humV1=VLh) from 3964-4284)**

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1 CTAGCTTTTT GCAAAAGCCT AGGCCTCCAA AAAAGCCTCC TCACTACTTC
51 TGGAAATAGCT CAGAGGCCGA GGCGGCCTCG GCCTCTGCAT AAATAAAAAA
101 AATTAGTCAG CCATGGGGCG GAGAATGGGC GGAAGTGGGC GGAGTTAGGG
20 151 GCGGGATGGG CGGAGTTAGG GGCGGGACTA TGGTTGCTGA CTAATTGAGA
201 TGCATGCTTT GCATACTTCT GCCTGCTGGG GAGCCTGGTT GCTGACTAAT
251 TGAGATGCAT GCTTTGCATA CTTCTGCCTG CTGGGGAGCC TGGGGACTTT
301 CCACACCCTA ACTGACACAC ATTCCACAGC TGCCTCGCGC GTTTCGGTGA
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25 401 GTCTGTAAGC GGATGCCGGG AGCAGACAAG CCCGTCAGGG CGCGTCAGCG
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30 651 GTCGTTGCGC TCGGCGGAGC GGTATCAGCT CACTCAAAGG CGGTAATACG
701 GTTATCCACA GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAG
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35 901 GAAGCTCCCT CGTGCCTCTT CCTGTTCCGA CCCTGCCGCT TACCGGATAC
951 CTGTCCGCCT TTCTCCCTTC GGAAGCGTG GCGCTTCTC ATAGCTCACG

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	1101	CGTCTTGAGT	CCAACCCGGT	AAGACACGAC	TTATCGCCAC	TGGCAGCAGC
	1151	CACTGGTAAC	AGGATTAGCA	GAGCGAGGTA	TGTAGGCGGT	GCTACAGAGT
5	1201	TCTTGAAGTG	GTGGCCTAAC	TACGGCTACA	CTAGAAGGAC	AGTATTTGGT
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	1301	TTGATCCGGC	AAACAAACCA	CCGCTGGTAG	CGGTGGTTTT	TTTGTGTTGCA
	1351	AGCAGCAGAT	TACGCGCAGA	AAAAAAGGAT	CTCAAGAAGA	TCCTTTGATC
	1401	TTTTCTACGG	GGTCTGACGC	TCAGTGGAAC	GAAAACTCAC	GTAAAGGGAT
10	1451	TTTGGTCATG	AGATTATCAA	AAAGGATCTT	CACCTAGATC	CTTTTAAATT
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	1551	GACAGTTACC	AATGCTTAAT	CAGTGAGGCA	CCTATCTCAG	CGATCTGTCT
	1601	ATTTTCGTTCA	TCCATAGTTG	CCTGACTCCC	CGTCGTGTAG	ATAACTACGA
	1651	TACGGGAGGG	CTTACCATCT	GGCCCCAGTG	CTGCAATGAT	ACCGCGAGAC
15	1701	CCACGCTCAC	CGGCTCCAGA	TTTATCAGCA	ATAAACCAGC	CAGCCGGAAG
	1751	GGCCGAGCGC	AGAAGTGGTC	CTGCAACTTT	ATCCGCTTCC	ATCCAGTCTA
	1801	TTAATTGTTG	CCGGGAAGCT	AGAGTAAGTA	GTTCGCCAGT	TAATAGTTTG
	1851	CGCAACGTTG	TTGCCATTGC	TGCAGGCATC	GTGGTGTCAC	GCTCGTCGTT
	1901	TGGTATGGCT	TCATTCAGCT	CCGGTTCCCA	ACGATCAAGG	CGAGTTACAT
20	1951	GATCCCCCAT	GTTGTGCAAA	AAAGCGGTTA	GCTCCTTCGG	TCCTCCGATC
	2001	GTTGTCAGAA	GTAAGTTGGC	CGCAGTGTTA	TCACTCATGG	TTATGGCAGC
	2051	ACTGCATAAT	TCTCTTACTG	TCATGCCATC	CGTAAGATGC	TTTTCTGTGA
	2101	CTGGTGAGTA	CTCAACCAAG	TCATTCTGAG	AATAGTGTAT	GCGGCGACCG
	2151	AGTTGCTCTT	GCCCGGCGTC	AACACGGGAT	AATACCGCGC	CACATAGCAG
25	2201	AACTTTAAAA	GTGCTCATCA	TTGGAAAACG	TTCTTCGGGG	CGAAAACTCT
	2251	CAAGGATCTT	ACCGCTGTTG	AGATCCAGTT	CGATGTAACC	CACTCGTGCA
	2301	CCCAACTGAT	CTTCAGCATC	TTTTACTTTC	ACCAGCGTTT	CTGGGTGAGC
	2351	AAAAACAGGA	AGGCAAAATG	CCGCAAAAAA	GGAATAAGG	GCGACACGGA
	2401	AATGTTGAAT	ACTCATACTC	TTCTTTTTC	AATATTATTG	AAGCATTTAT
30	2451	CAGGGTTATT	GTCTCATGAG	CGGATACATA	TTTGAATGTA	TTTAGAAAAA
	2501	TAAACAAATA	GGGGTTCCGC	GCACATTTC	CCGAAAAGTG	CCACCTGACG
	2551	TCTAAGAAAC	CATTATTATC	ATGACATTAA	CCTATAAAAA	TAGGCGTATC
	2601	ACGAGGCCCT	TTCGTCTTCA	AGAATTCAGC	TTGGCTGCAG	TGAATAATAA
	2651	AATGTGTGTT	TGTCCGAAAT	ACGCGTTTTG	AGATTTCTGT	CGCCGACTAA
35	2701	ATTCATGTCG	CGCGATAGTG	GTGTTTATCG	CCGATAGAGA	TGGCGATATT
	2751	GGAAAAATCG	ATATTTGAAA	ATATGGCATA	TTGAAAATGT	CGCCGATGTG
	2801	AGTTTCTGTG	TAACATGATAT	CGCCATTTTT	CCAAAAGTGA	TTTTTGGGCA

	2851	TACGCGATAT	CTGGCGATAG	CGCTTATATC	GTTTACGGGG	GATGGCGATA
	2901	GACGACTTTG	GTGACTTGGG	CGATTCTGTG	TGTCGCAAAT	ATCGCAGTTT
	2951	CGATATAGGT	GACAGACGAT	ATGAGGCTAT	ATCGCCGATA	GAGGCGACAT
	3001	CAAGCTGGCA	CATGGCCAAT	GCATATCGAT	CTATACATTG	AATCAATATT
5	3051	GGCCATTAGC	CATATTATTC	ATTGGTTATA	TAGCATAAAT	CAATATTGGC
	3101	TATTGGCCAT	TGCATACGTT	GTATCCATAT	CATAATATGT	ACATTTATAT
	3151	TGGCTCATGT	CCAACATTAC	CGCCATGTTG	ACATTGATTA	TTGACTAGTT
	3201	ATTAATAGTA	ATCAATTACG	GGGTCATTAG	TTCATAGCCC	ATATATGGAG
	3251	TTCCGCGTTA	CATAACTTAC	GGTAAATGGC	CCGCCTGGCT	GACCGCCCAA
10	3301	CGACCCCCGC	CCATTGACGT	CAATAATGAC	GTATGTTCCC	ATAGTAACGC
	3351	CAATAGGGAC	TTTCCATTGA	CGTCAATGGG	TGGAGTATTT	ACGGTAAACT
	3401	GCCCCACTTG	CAGTACATCA	AGTGTATCAT	ATGCCAAGTA	CGCCCCCTAT
	3451	TGACGTCAAT	GACGGTAAAT	GGCCCGCCTG	GCATTATGCC	CAGTACATGA
	3501	CCTTATGGGA	CTTTCCTACT	TGGCAGTACA	TCTACGTATT	AGTCATCGCT
15	3551	ATTACCATGG	TGATGCGGTT	TTGGCAGTAC	ATCAATGGGC	GTGGATAGCG
	3601	GTTTGACTCA	CGGGGATTTT	CAAGTCTCCA	CCCCATTGAC	GTCAATGGGA
	3651	GTTTGTTTTG	GCACCAAAT	CAACGGGACT	TTCCAAAATG	TCGTAACAAC
	3701	TCCGCCCCAT	TGACGCAAAT	GGGCGGTAGG	CGTGTACGGT	GGGAGGTCTA
	3751	TATAAGCAGA	GCTCGTTTAG	TGAACCGTCA	GATCGCCTGG	AGACGCCATC
20	3801	CACGCTGTTT	TGACCTCCAT	AGAAGACACC	GGGACCGATC	CAGCCTCCGC
	3851	AAGCTTGATA	TCGAATTCCT	GCAGCCCGGG	GGATCCGCCC	GCTTGCCGCC
	3901	ACCATGGAGA	CCCCGCCCCA	GCTGCTGTTC	CTGCTGCTGC	TGTGGCTGCC
	3951	CGACACCACC	GGCGACATTC	TGCTGACCCA	GTCTCCAGCC	ACCCTGTCTC
	4001	TGAGTCCAGG	AGAAAGAGCC	ACTCTCTCCT	GCAGGGCCAG	TCAGAACATT
25	4051	GGCACAAGCA	TACAGTGGTA	TCAACAAAAA	CCAGGTCAGG	CTCCAAGGCT
	4101	TCTCATAAGG	TCTTCTTCTG	AGTCTATCTC	TGGGATCCCT	TCCAGGTTTA
	4151	GTGGCAGTGG	ATCAGGGACA	GATTTTACTC	TTACCATCAG	CAGTCTGGAG
	4201	CCTGAAGATT	TTGCAGTGTA	TTACTGTCAA	CAAAGTAATA	CCTGGCCATT
	4251	CACGTTTCGGC	CAGGGGACCA	AGCTGGAGAT	CAAACGTGAG	TATTCTAGAA
30	4301	AGATCCTAGA	ATTCTAAACT	CTGAGGGGGT	CGGATGACGT	GGCCATTCTT
	4351	TGCCTAAAGC	ATTGAGTTTA	CTGCAAGGTC	AGAAAAGCAT	GCAAAGCCCT
	4401	CAGAATGGCT	GCAAAGAGCT	CCAACAAAAC	AATTTAGAAC	TTTATTAAGG
	4451	AATAGGGGGA	AGCTAGGAAG	AAACTCAAAA	CATCAAGATT	TTAAATACGC
	4501	TTCTTGGTCT	CCTTGCTATA	ATTATCTGGG	ATAAGCATGC	TGTTTTCTGT
35	4551	CTGTCCCTAA	CATGCCCTGT	GATTATCCGC	AAACAACACA	CCCAAGGGCA
	4601	GAACTTTGTT	ACTTAAACAC	CATCCTGTTT	GCTTCTTTCC	TCAGGAAGTG
	4651	TGGCTGCACC	ATCTGTCTTC	ATCTTCCCGC	CATCTGATGA	GCAGTTGAAA

4701	TCTGGA	ACTG	CCTCTG	TGTGT	GTGCCT	GCTG	AATAACT	TCT	ATCCCAG	GAGA	
4751	GGCCAA	AGTA	CAGTGG	AAGG	TGGATA	AACGC	CCTCCA	ATCG	GGTAACT	CCC	
4801	AGGAGAG	TGT	CACAGAG	CAG	GACAGCA	AAGG	ACAGCAC	CTA	CAGCCTC	CAGC	
4851	AGCACCC	TGA	CGCTGAG	CAA	AGCAGACT	TAC	GAGAAAC	CACA	AAGTCTA	CGC	
5	4901	CTGCGA	AAGTC	ACCCAT	CAGG	GCCTGAG	CTC	GCCCCGT	CACA	AAGAGCT	TCA
	4951	ACAGGGG	GAGA	GTGTTAG	AGG	GAGAAGT	GCC	CCCACCT	GCT	CCTCAGT	TCC
	5001	AGCCTGA	CCC	CCTCCC	ATCC	TTTGGC	CTCT	GACCCTT	TTTT	CCACAGG	GGGA
	5051	CCTACCC	CTA	TTGCGGT	CCCT	CCAGCTC	ATC	TTTCAC	CTCA	CCCCCCT	CCT
	5101	CCTCCTT	GGC	TTTAATT	TATG	CTAATGT	TGG	AGGAGA	AATGA	ATAAAATA	AAAG
10	5151	TGAATCT	TTTG	CACCTGT	GGT	TTCTCTC	TTT	CCTCAT	TTAA	TAATTATT	TAT
	5201	CTGTTGT	TTTA	CCAAC	TACTC	AATTTCT	CTT	ATAAGG	GACT	AAATATG	TAG
	5251	TCATCCT	AAG	GCGCATA	AACC	ATTTATA	AAAA	ATCATC	CCTTC	ATTCTAT	TTTT
	5301	ACCCTAT	CAT	CCTCTG	CAAG	ACAGTC	CCTCC	CTCAA	ACCCA	CAAGCCT	TCT
	5351	GTCCTCA	CAG	TCCCCT	GGGC	CATGGT	AGGA	GAGACT	TGCT	TCCTTGT	TTTT
15	5401	CCCCTC	CCTCA	GCAAGCC	CCTC	ATAGTC	CCTTT	TTAAGG	GGA	CAGGTCT	TAC
	5451	AGTCATA	TAT	CCTTTG	ATTTC	AATTC	CCTGA	GAATCA	ACCA	AAGCAA	ATTT
	5501	TTCAAA	AGAA	GAAACCT	GCT	ATAAAG	AGAA	TCATT	CATTG	CAACAT	GATA
	5551	TAAAA	TAACA	ACACA	ATAAA	AGCAAT	TAAA	TAAACA	AAACA	ATAGGG	AAAT
	5601	GTTTA	AGTTC	ATCATG	GTAC	TTAGACT	TTAA	TGGAAT	GTCA	TGCCTT	ATTT
20	5651	ACATTTT	TAA	ACAGGT	ACTG	AGGGACT	CCT	GTCTG	CCAAG	GGCCGT	ATTG
	5701	AGTACTT	TCC	ACAAC	CTAAT	TTAAT	CCACA	CTATA	CTGTG	AGATTAA	AAAA
	5751	CATTCAT	TAA	AATGTT	GCAA	AGGTT	CTATA	AAGCT	GAGAG	ACAAAT	ATAT
	5801	TCTATA	ACTC	AGCAAT	CCCA	CTTCT	AGATG	ACTG	AGTGT	CCCACCC	ACC
	5851	AAAAAA	ACTAT	GCAAGA	ATGT	TCAAAG	CAGC	TTTATT	TTACA	AAAGCC	AAAA
25	5901	ATTGGAA	ATA	GCCCG	ATTGT	CCAACA	ATAG	AATG	AGTTAT	TAAACT	GTGG
	5951	TATGTTT	TATA	CATTAGA	ATA	CCCAAT	GAGG	AGAAT	TAACA	AGCTACA	ACT
	6001	ATACCTA	CTC	ACACAG	ATGA	ATCTCA	TAAA	AATAAT	GTTA	CATAAG	AGAA
	6051	ACTCAAT	GCA	AAAGAT	ATGT	TCTGT	ATGTT	TTCAT	CCATA	TAAAGT	TCAA
	6101	AACCAGG	TAA	AAATAA	AGTT	AGAAAT	TTGG	ATGGAA	ATTA	CTCTTAG	CTG
30	6151	GGGGT	GCGC	AGTTAG	TGCC	TGGGAG	AAGA	CAAGA	AGGGG	CTTCTG	GGGGT
	6201	CTTGGT	AATG	TTCTGT	TCCT	CGTGT	GGGG	TGTGC	AGTTA	TGATCT	GTGC
	6251	ACTGTT	CTGT	ATACAC	ATTA	TGCTT	CAAAA	TAACT	TCACA	TAAAGA	ACAT
	6301	CTTATA	CCCA	GTTAAT	AGAT	AGAAG	AGGAA	TAAGT	AATAG	GTCAAG	ACCA
	6351	CGCAG	CTGGT	AAGTGG	GGGG	GCCTG	GGATC	AAATAG	CTAC	CTGCCTA	ATC
35	6401	CTGCC	CTCTT	GAGCC	CTGAA	TGAGT	CTGCC	TTCCAG	GGGCT	CAAGGT	GTCTC
	6451	AACAAA	ACAA	CAGGC	CTGCT	ATTTTC	CCTGG	CATCT	GTGCC	CTGTTT	TGGCT
	6501	AGCTAG	GAGC	ACACAT	ACAT	AGAAAT	TAAA	TGAAAC	AGAC	CTTCAG	CAAG

6551 GGGACAGAGG ACAGAATTAA CCTTGCCCAG ACACTGGAAA CCCATGTATG
 6601 AACACTCACA TGTTTGGGAA GGGGGAAGGG CACATGTAAA TGAGGACTCT
 6651 TCCTCATTCT ATGGGGCACT CTGGCCCTGC CCCTCTCAGC TACTCATCCA
 6701 TCCAACACAC CTTTCTAAGT ACCTCTCTCT GCCTACACTC TGAAGGGGTT
 5 6751 CAGGAGTAAC TAACACAGCA TCCCTTCCCT CAAATGACTG ACAATCCCTT
 6801 TGTCTTGCTT TGTTTTTCTT TCCAGTCAGT ACTGGGAAAG TGGGGAAGGA
 6851 CAGTCATGGA GAAACTACAT AAGGAAGCAC CTTGCCCTTC TGCCTCTTGA
 6901 GAATGTTGAT GAGTATCAAA TCTTTCAAAC TTTGGAGGTT TGAGTAGGGG
 6951 TGAGACTCAG TAATGTCCCT TCCAATGACA TGAAGTTGCT CACTCATCCC
 10 7001 TGGGGGCCAA ATTGAACAAT CAAAGGCAGG CATAATCCAG CTATGAATTC
 7051 TAGGATCGAT CCAGACATGA TAAGATACAT TGATGAGTTT GGACAAACCA
 7101 CAACTAGAAT GCAGTGAAAA AAATGCTTTA TTTGTGAAAT TTGTGATGCT
 7151 ATTGCTTTAT TTGTAACCAT TATAAGCTGC AATAAACAAAG TTAACAACAA
 7201 CAATTGCATT CATTTTATGT TTCAGGTTCA GGGGGAGGTG TGGGAGGTTT
 15 7251 TTTAAAGCAA GTAAAACCTC TACAAATGTG GTATGGCTGA TTATGATCTC
 7301 TAGTCAAGGC ACTATACATC AAATATTCCT TATTAACCCC TTTACAAATT
 7351 AAAAAGCTAA AGGTACACAA TTTTGTAGCA TAGTTATTAA TAGCAGACAC
 7401 TCTATGCCTG TGTGGAGTAA GAAAAAACAG TATGTTATGA TTATAACTGT
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 20 7501 AGCAGTGCAG CTTTTTCCTT TGTGGTGTA AATAGCAAAGC AAGCAAGAGT
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 7651 TGAGAGTCAG CAGTAGCCTC ATCATCACTA GATGGCATT CTTCTGAGCA
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 25 7751 TTCCATAGGT TGGAATCTAA AATACACAAA CAATTAGAAT CAGTAGTTTA
 7801 ACACATTATA CACTTAAAAA TTTTATATTT ACCTTAGAGC TTTAAATCTC
 7851 TGTAGGTAGT TTGTCCAATT ATGTCACACC ACAGAAGTAA GGTTCCTTCA
 7901 CAAAGATCCG GGACCAAAGC GGCCATCGTG CCTCCCCACT CCTGCAGTTC
 7951 GGGGGCATGG ATGCGCGGAT AGCCGCTGCT GGTTCCTGG ATGCCGACGG
 30 8001 ATTTGCACTG CCGGTAGAAC TCCGCGAGGT CGTCCAGCCT CAGGCAGCAG
 8051 CTGAACCAAC TCGCGAGGGG ATCGAGCCCG GGGTGGGCGA AGAACTCCAG
 8101 CATGAGATCC CCGCGCTGGA GGATCATCCA GCCGGCGTCC CGGAAAACGA
 8151 TTCCGAAGCC CAACCTTTCA TAGAAGGCGG CGGTGGAATC GAAATCTCGT
 8201 GATGGCAGGT TGGGCGTCGC TTGGTCGGTC ATTTGGAACC CCAGAGTCCC
 35 8251 GCTCAGAAGA ACTCGTCAAG AAGGCGATAG AAGGCGATGC GCTGCGAATC
 8301 GGGAGCGGCG ATACCGTAAA GCACGAGGAA GCGGTCAGCC CATTCGCCCG
 8351 CAAGCTCTTC AGCAATATCA CGGGTAGCCA ACGCTATGTC CTGATAGCGG

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8401  TCCGCCACAC  CCAGCCGGCC  ACAGTCGATG  AATCCAGAAA  AGCGGCCATT
8451  TTCCACCATG  ATATTCGGCA  AGCAGGCATC  GCCATGGGTC  ACGACGAGAT
8501  CCTCGCCGTC  GGGCATGCGC  GCCTTGAGCC  TGGCGAACAG  TTCGGCTGGC
8551  GCGAGCCCCT  GATGCTCTTC  GTCCAGATCA  TCCTGATCGA  CAAGACCGGC
5    8601  TTCCATCCGA  GTACGTGCTC  GCTCGATGCG  ATGTTTCGCT  TGGTGGTCGA
8651  ATGGGCAGGT  AGCCGGATCA  AGCGTATGCA  GCCGCCGCAT  TGCATCAGCC
8701  ATGATGGATA  CTTTCTCGGC  AGGAGCAAGG  TGAGATGACA  GGAGATCCTG
8751  CCCCggCACT  TCGCCCAATA  GCAGCCAGTC  CCTTCCCGCT  TCAGTGACAA
8801  CGTCGAGCAC  AGCTGCGCAA  GGAACGCCCG  TCGTGGCCAG  CCACGATAGC
10   8851  CGCGCTGCCT  CGTCCTGCAG  TTCATTCAGG  GCACCGGACA  GGTCGGTCTT
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8951  CAGAGCAGCC  GATTGTCTGT  TGTGCCCAGT  CATAGCCGAA  TAGCCTCTCC
9001  ACCCAAGCGG  CCGGAGAACC  TCGGTGCAAT  CCATCTTGTT  CAATCATGCG
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15   9101  AGATCCTTGG  CGGCAAGAAA  GCCATCCAGT  TTACTTTGCA  GGGCTTCCCA
9151  ACCTTACCAG  AGGGCGCCCC  AGCTGGCAAT  TCCGGTTCGC  TTGCTGTCCA
9201  TAAAACCGCC  CAGTCTAGCT  ATCGCCATGT  AAGCCCACTG  CAAGCTACCT
9251  GCTTTCTCTT  TCGCTTGCG  TTTTCCCTTG  TCCAGATAGC  CCAGTAGCTG
9301  ACATTTCATC  GGGGTCAGCA  CCGTTTCTGC  GGACTGGCTT  TCTACGTGTT
20   9351  CCGCTTCCTT  TAGCAGCCCT  TGCGCCCTGA  GTGCTTGCGG  CAGCGTGAAG

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SEQ ID NO:18**Nucleotide sequence of the expression vector HCMV-K HuAb-VL1 hum V2****(Complete DNA Sequence of a humanised light chain expression vector comprising****25 SEQ ID NO: 13 (humV2=VLm) from 3926-4246)**

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1    CTAGCTTTTT  GCAAAAGCCT  AGGCCTCCAA  AAAAGCCTCC  TCACTACTTC
51   TGGAATAGCT  CAGAGGCCGA  GGCGGCCTCG  GCCTCTGCAT  AAATAAAAAA
101  AATTAGTCAG  CCATGGGGCG  GAGAATGGGC  GGAAGTGGGC  GGAGTTAGGG
30   151  GCGGGATGGG  CGGAGTTAGG  GGCGGGACTA  TGGTTGCTGA  CTAATTGAGA
201  TGCATGCTTT  GCATACTTCT  GCCTGCTGGG  GAGCCTGGTT  GCTGACTAAT
251  TGAGATGCAT  GCTTTGCATA  CTTCTGCCTG  CTGGGGAGCC  TGGGGACTTT
301  CCACACCCTA  ACTGACACAC  ATTCCACAGC  TGCCTCGCGC  GTTTCGGTGA
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35   401  GTCTGTAAGC  GGATGCCGGG  AGCAGACAAG  CCCGTCAGGG  CGCGTCAGCG
451  GGTGTTGGCG  GGTGTCGGGG  CGCAGCCATG  ACCCAGTCAC  GTAGCGATAG

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501 CGGAGTGTAT ACTGGCTTAA CTATGCGGCA TCAGAGCAGA TTGTACTGAG
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 601 ACCGCATCAG GCGCTCTTCC GCTTCCTCGC TCACTGACTC GCTGCGCTCG
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 5 701 GTTATCCACA GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAAG
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 20 1451 TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC CTTTTAAATT
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 25 1701 CCACGCTCAC CGGCTCCAGA TTTATCAGCA ATAAACCAGC CAGCCGGAAG
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 30 1951 GATCCCCCAT GTTGTGCAAA AAAGCGGTTA GCTCCTTCGG TCCTCCGATC
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 35 2201 AACTTTAAAA GTGCTCATCA TTGGAACG TTCTTCGGGG CGAAAACTCT
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 2301 CCCAACTGAT CTTAGCATC TTTTACTTTC ACCAGCGTTT CTGGGTGAGC

	2351	AAAAACAGGA	AGGCAAAATG	CCGCAAAAAA	GGGAATAAGG	GCGACACGGA
	2401	AATGTTGAAT	ACTCATACTC	TTCCTTTTTT	AATATTATTG	AAGCATTAT
	2451	CAGGGTTATT	GTCTCATGAG	CGGATACATA	TTTGAATGTA	TTTAGAAAAA
	2501	TAAACAAATA	GGGGTTCCGC	GCACATTTC	CCGAAAAGTG	CCACCTGACG
5	2551	TCTAAGAAAC	CATTATTATC	ATGACATTAA	CCTATAAAAA	TAGGCGTATC
	2601	ACGAGGCCCT	TTCGTCTTCA	AGAATTCAGC	TTGGCTGCAG	TGAATAATAA
	2651	AATGTGTGTT	TGTCCGAAAT	ACGCGTTTTG	AGATTTCTGT	CGCCGACTAA
	2701	ATTCATGTCG	CGCGATAGTG	GTGTTTATCG	CCGATAGAGA	TGGCGATATT
	2751	GGAAAAATCG	ATATTTGAAA	ATATGGCATA	TTGAAAATGT	CGCCGATGTG
10	2801	AGTTTCTGTG	TAAGTGATAT	CGCCATTTTT	CCAAAAGTGA	TTTTTGGGCA
	2851	TACGCGATAT	CTGGCGATAG	CGCTTATATC	GTTTACGGGG	GATGGCGATA
	2901	GACGACTTTG	GTGACTTGGG	CGATTCTGTG	TGTCGCAAAT	ATCGCAGTTT
	2951	CGATATAGGT	GACAGACGAT	ATGAGGCTAT	ATCGCCGATA	GAGGCGACAT
	3001	CAAGCTGGCA	CATGGCCAAT	GCATATCGAT	CTATACATTG	AATCAATATT
15	3051	GGCCATTAGC	CATATTATTC	ATTGGTTATA	TAGCATAAAAT	CAATATTGGC
	3101	TATTGGCCAT	TGCATACGTT	GTATCCATAT	CATAATATGT	ACATTTATAT
	3151	TGGCTCATGT	CCAACATTAC	CGCCATGTTG	ACATTGATTA	TTGACTAGTT
	3201	ATTAATAGTA	ATCAATTACG	GGGTCATTAG	TTCATAGCCC	ATATATGGAG
	3251	TTCCGCGTTA	CATAACTTAC	GGTAAATGGC	CCGCCTGGCT	GACCGCCCAA
20	3301	CGACCCCCGC	CCATTGACGT	CAATAATGAC	GTATGTTCCC	ATAGTAACGC
	3351	CAATAGGGAC	TTTCCATTGA	CGTCAATGGG	TGGAGTATTT	ACGGTAAACT
	3401	GCCCCACTTG	CAGTACATCA	AGTGTATCAT	ATGCCAAGTA	CGCCCCCTAT
	3451	TGACGTCAAT	GACGGTAAAT	GGCCCGCCTG	GCATTATGCC	CAGTACATGA
	3501	CCTTATGGGA	CTTTCCTACT	TGGCAGTACA	TCTACGTATT	AGTCATCGCT
25	3551	ATTACCATGG	TGATGCGGTT	TTGGCAGTAC	ATCAATGGGC	GTGGATAGCG
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	3651	GTTTGTTTTG	GCACCAAAAT	CAACGGGACT	TTCCAAAATG	TCGTAACAAC
	3701	TCCGCCCCAT	TGACGCAAAT	GGGCGGTAGG	CGTGTACGGT	GGGAGGTCTA
	3751	TATAAGCAGA	GCTCGTTTAG	TGAACCGTCA	GATCGCCTGG	AGACGCCATC
30	3801	CACGCTGTTT	TGACCTCCAT	AGAAGACACC	GGGACCGATC	CAGCCTCCGC
	3851	AAGCTTGCCG	CCACCATGGA	GACCCCCGCC	CAGCTGCTGT	TCCTGCTGCT
	3901	GCTGTGGCTG	CCCGACACCA	CCGGCGACAT	TCTGCTGACC	CAGTCTCCAG
	3951	CCACCCTGTC	TCTGAGTCCA	GGAGAAAAGAG	CCACTTTCTC	CTGCAGGGCC
	4001	AGTCAGAACA	TTGGCACAAAG	CATACAGTGG	TATCAACAAA	AAACAAATGG
35	4051	TGCTCCAAGG	CTTCTCATAA	GGTCTTCTTC	TGAGTCTATC	TCTGGGATCC
	4101	CTTCCAGGTT	TAGTGGCAGT	GGATCAGGGA	CAGATTTTAC	TCTTACCATC
	4151	AGCAGTCTGG	AGCCTGAAGA	TTTTGCAGTG	TATTACTGTC	AACAAAGTAA

	4201	TACCTGGCCA	TTCACGTTCTG	GCCAGGGGAC	CAAGCTGGAG	ATCAAACGTG
	4251	AGTATTCTAG	AAAGATCCTA	GAATTCTAAA	CTCTGAGGGG	GTCGGATGAC
	4301	GTGGCCATTC	TTTGCCTAAA	GCATTGAGTT	TACTGCAAGG	TCAGAAAAGC
	4351	ATGCAAAGCC	CTCAGAATGG	CTGCAAAGAG	CTCCAACAAA	ACAATTTAGA
5	4401	ACTTTATTAA	GGAATAGGGG	GAAGCTAGGA	AGAAACTCAA	AACATCAAGA
	4451	TTTTAAATAC	GCTTCTTGGT	CTCCTTGCTA	TAATTATCTG	GGATAAGCAT
	4501	GCTGTTTTCT	GTCTGTCCCT	AACATGCCCT	GTGATTATCC	GCAAACAACA
	4551	CACCCAAGGG	CAGAACTTTG	TTACTTAAAC	ACCATCCTGT	TTGCTTCTTT
	4601	CCTCAGGAAC	TGTGGCTGCA	CCATCTGTCT	TCATCTTCCC	GCCATCTGAT
10	4651	GAGCAGTTGA	AATCTGGAAC	TGCCTCTGTT	GTGTGCCTGC	TGAATAACTT
	4701	CTATCCCAGA	GAGGCCAAAG	TACAGTGGAA	GGTGGATAAC	GCCCTCCAAT
	4751	CGGGTAACTC	CCAGGAGAGT	GTCACAGAGC	AGGACAGCAA	GGACAGCACC
	4801	TACAGCCTCA	GCAGCACCTT	GACGCTGAGC	AAAGCAGACT	ACGAGAAACA
	4851	CAAAGTCTAC	GCCTGCGAAG	TCACCCATCA	GGGCCTGAGC	TCGCCCGTCA
15	4901	CAAAGAGCTT	CAACAGGGGA	GAGTGTTAGA	GGGAGAAAGT	CCCCCACCTG
	4951	CTCCTCAGTT	CCAGCCTGAC	CCCCTCCCAT	CCTTTGGCCT	CTGACCCTTT
	5001	TTCCACAGGG	GACCTACCCC	TATTGCGGTC	CTCCAGCTCA	TCTTTACCT
	5051	CACCCCCCTC	CTCCTCCTTG	GCTTTAATTA	TGCTAATGTT	GGAGGAGAAT
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20	5151	AATAATTATT	ATCTGTTGTT	TACCAACTAC	TCAATTTCTC	TTATAAGGGA
	5201	CTAAATATGT	AGTCATCCTA	AGGCGCATAA	CCATTTATAA	AAATCATCCT
	5251	TCATTCTATT	TTACCCTATC	ATCCTCTGCA	AGACAGTCCT	CCCTCAAACC
	5301	CACAAGCCTT	CTGTCCTCAC	AGTCCCCTGG	GCCATGGTAG	GAGAGACTTG
	5351	CTTCCTTGTT	TTCCCCTCCT	CAGCAAGCCC	TCATAGTCCT	TTTTAAGGGT
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	5451	CAAAGCAAAT	TTTTCAAAAG	AAGAAACCTG	CTATAAAGAG	AATCATTTCAT
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5	6251	CATAAAGAAC	ATCTTATACC	CAGTTAATAG	ATAGAAGAGG	AATAAGTAAT
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	6351	ACCTGCCTAA	TCCTGCCCTC	TTGAGCCCTG	AATGAGTCTG	CCTTCCAGGG
	6401	CTCAAGGTGC	TCAACAAAC	AACAGGCCTG	CTATTTTCCT	GGCATCTGTG
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10	6501	ACCTTCAGCA	AGGGGACAGA	GGACAGAATT	AACCTTGCCC	AGACACTGGA
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	6601	AATGAGGACT	CTTCCTCATT	CTATGGGGCA	CTCTGGCCCT	CCCCCTCTCA
	6651	GCTACTCATC	CATCCAACAC	ACCTTTCTAA	GTACCTCTCT	CTGCCTACAC
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15	6751	TGACAAATCCC	TTTGTCTGCT	TTTGTTTTTC	TTTCCAGTCA	GTACTGGGAA
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	7151	AGTTAACAAC	AACAATTGCA	TTCATTTTAT	GTTTCAGGTT	CAGGGGGAGG
	7201	TGTGGGAGGT	TTTTTAAAGC	AAGTAAAACC	TCTACAAATG	TGGTATGGCT
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35	7751	ATCAGTAGTT	TAACACATTA	TACACTTAAA	AATTTTATAT	TTACCTTAGA
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15 8601 CTTGGTGGTC GAATGGGCAG GTAGCCGGAT CAAGCGTATG CAGCCGCCGC
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25 9101 CAGGGCTTCC CAACCTTACC AGAGGGCGCC CCAGCTGGCA ATTCCGGTTC
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9301 TTTCTACGTG TTCCGCTTCC TTTAGCAGCC CTTGCGCCCT GAGTGCTTGC
30 9351 GGCAGCGTGA AG

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Example 9: In vitro efficacy of CD45RO/RB binding humanised antibodies

To determine the efficacy of the CD45RO/RB binding humanised antibodies VHE/humV1 and
 35 VHQ/humV1 in comparison to the chimeric antibody the ability to induce apoptosis in human
 T cells and also the ability to inhibit human T cell proliferation is analysed.

Cells and reagents

Peripheral blood mononuclear cells (PBMC) are isolated from leukopheresis samples of healthy human donors with known blood type, but unknown HLA type by centrifugation over Ficoll-Hypaque (Pharmacia LKB). PBMC used as stimulators are first depleted of T and NK cells by using CD3-coated ferromagnetic beads (Miltenyi). Beads and contaminating cells are removed by magnetic field. T cell-depleted PBMC are used as stimulator cells after irradiation (50 Gy). CD4⁺ T cells are used as responder cells in MLR and are isolated from PBMC with a CD4 T cell negative selection kit (Miltenyi).

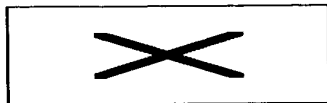
The obtained cells are analyzed by FACScan or FACSCalibur (Becton Dickinson & Co., CA) and the purity of the obtained cells is >75%. Cells are suspended in RPMI1640 medium supplemented with 10% heat-inactivated FCS, penicillin, streptomycin and L-glutamine.

Apoptosis assays

Human PBMC of three healthy voluntary donors are cultured in growth medium (RPMI1640+10%FCS) overnight (<16h) in the presence of CD45R0/RB binding chimeric mAb, humanized antibodies (VHE/humV1 and VHQ/humV1) or anti-LPS control mAb. If indicated, a cross-linking reagent, F(ab')₂-fragment of goat anti-human IgG (Cat.No. 109-006-098, JacksonLab) is included at a µg/ml concentration being twice as high as the sample's anti-CD45 antibodies concentration. The PBS-concentration in all wells introduced by the antibody reagents is kept constant among all samples, namely at 20% (v/v) for samples without cross-linker or at 40% (v/v) for samples with cross-linker. Earlier experiments demonstrate that the amount of PBS does not affect the readout.

After overnight culture in the presence of the antibodies, the samples are subjected to flow cytometry analyses and stained with the apoptosis marker AnnexinV-FITC (Cat.No. 556419, BD/Pharmingen) and the T cell marker CD2-PE (Cat.No. 556609, BD/Pharmingen). The samples are run in a Becton Dickinson FACSCalibur instrument and the data are analyzed using the CellQuest Pro Software.

From the data collected, curves are fitted using the software Origin v7.0300 The equation used for fitting is



("Sigmoid-Logistic")

5

A_1 : final value (for fitting sessions set to "shared " and "floating")

A_2 : initial value (for fitting sessions set to "shared " and "floating")

p : power

X_0 : ED_{50} ; IC_{50} (see below).

10

In the absence of cross-linker, VHE/humV1 is most effective, with an ED_{50} value of 148 ± 71 nM, followed by VHQ/humV1 with 377 ± 219 nM. CD45R0/RB binding chimeric antibody is less effective with an ED_{50} value of 2440 ± 1205 nM.

15

In the presence of a cross-linking antiserum, the ED_{50} values are shifted dramatically towards higher efficacy by at least two orders of magnitude. In addition, the presence of cross-linker permitted higher levels of apoptosis at very high antibody concentrations, now reaching up to 80 %, whereas the absence of cross-linker only allowed for up to 50% of apoptosis. In the presence of cross-linker, the curves (antibody concentration / % apoptosis) are bi-modal with two plateaus: the first plateau is reached at low antibody concentrations (~ 5 nM), where the apoptosis level corresponds to the maximum level obtained in the absence of cross-linker. The second plateau is reached at high antibody concentrations (~ 500 nM) and apoptosis is observed within 70-80% of the T cell population.

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Both CD45R0/RB binding humanised mAb are equally effective and better or equal compared to CD45R0/RB binding chimeric mAb with respect to their ability to induce apoptosis in primary human T cells.

Mixed Lymphocyte Reaction assays

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One $\times 10^5$ PBMC or 5×10^4 of $CD4^+$ cells are mixed with 1×10^5 or 5×10^4 T cells-depleted irradiated (50 Gy) PBMC in each well of 96-well culture plates in the presence or absence of the different concentrations of mAb.

The mixed cells are cultured for 5 days and proliferation is determined by pulsing the cells with ^3H -thymidine for the last 16 – 20 hours of culture. MLR inhibition at each antibody concentration is expressed as percentage inhibition as described in Example 2.

- 5 The effect of increasing concentrations of VHE/humV1 and VHQ/humV1 on MLR is evaluated in three responder:stimulator combinations. All antibodies inhibit the MLR in a dose-dependent manner. The IC_{50} values (see above) are similar for the humanized Ab VHE/humV1 (7 ± 7 nM) and VHQ/humV1 (39 ± 54 nM). Both humanised antibodies are more potent in inhibiting MLR than the parental chimeric antibody (IC_{50} of 347 ± 434 nM). As
10 usually seen with MLR experiments, donor variability is high in these experiments.

Example 10: Specificity of CD45RB/RO binding molecule

- The CD45 molecule is expressed on all leukocytes. However, different CD45 isoforms are
15 expressed by the various leukocyte subsets. In order to determine the leukocyte subset reactivity of CD45RB/RO binding chimeric antibody molecule immunofluorescent labeling of human leukocytes with subset-specific markers and simultaneous immunofluorescent labeling with a dye-conjugated CD45RB/RO binding chimeric antibody is performed, followed by flow cytometry analysis. Briefly, specific subsets of a freshly isolated preparation of
20 human peripheral blood mononuclear cells (PBMC), human platelets, human peripheral blood neutrophils or human bone-marrow derived hematopoietic stem cells are identified by incubation with phycoerythrin-coupled antibodies against CD2 (T lymphocytes), CD14 (monocytes), CD19 (B lymphocytes), CD34 (stem cells), CD42a (platelets), CD56 (natural killer cells) or CD66b (granulocytes). Simultaneous binding of a FITC-labeled chimeric
25 CD45RB/RO binding molecule is detected on T lymphocytes, monocytes, stem cells, natural killer cells and granulocytes, but not on platelets or B lymphocytes.

Example 11: In vitro induction of suppressor T cells (T regulatory cells) and of functionally paralyzed T cells

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To demonstrate the ability of a CD45RO/RB binding chimeric antibody to induce suppressor T cells, the antibody is included at various concentrations during the generation of CD8+ T cell lines reactive with the antigen matrix protein 1 (MP1) of hemophilus influenza. These lines are generated through repeated co-culture of CD8+ human lymphocytes with CD14+

human monocytes pulsed with the antigen. Later on, CD14+ monocytes can be replaced with a human leukocyte antigen-2 positive cell line as an MP1 antigen-presenting cell (APC). If such MP1-specific CD8+ T cells from a culture including CD45RO/RB binding chimeric antibody are mixed with freshly isolated human CD8+ T cells and this mixture of cells is stimulated with the MP1 antigen on APC, the addition of CD8+ T cells from the culture in the presence of CD45RO/RB binding molecule is able to reduce the IFN- γ production in an antibody-dose-dependent fashion. No CD45RO/RB binding chimeric antibody is present during this IFN- γ assay culture, indicating that the pre-treatment with the CD45RO/RB mAb has induced CD8+ T cells capable of suppressing the activation of freshly isolated T cells. Because of this induction of suppressor T regulatory cells by the CD45RO/RB binding chimeric antibody, the antibody may be useful in diseases, where a dysregulated and/or activated T cell population is thought to contribute to the pathology. Examples of such diseases include autoimmune diseases, transplant rejection, psoriasis, inflammatory bowel disease and allergies.

To demonstrate the ability of a chimeric CD45RO/RB binding molecule to render T cells hyporesponsive (anergic) to further stimulation, i.e. to functionally paralyze T cells, the antibody is included during the generation of CD8+ T cell lines reactive with the antigen matrix protein 1 (MP1) of hemophilus influenza as outlined above. Paralysis is assessed by activating the T cells (exposed prior to CD45RO/RB binding chimeric antibody) with MP1 antigen presented by APC. No CD45RO/RB binding molecule is present in this culture. CD8+ T cells not exposed to CD45RO/RB binding chimeric antibody previously produce IFN- γ upon the mentioned stimulus. In contrast, CD8+ T cells pre-treated with CD45RO/RB binding chimeric antibody show a markedly reduced to inexistent production of this cytokine in response to the antigen-stimulus, demonstrating the CD45RO/RB binding chimeric antibody's ability to functionally paralyze human T cells. Because of this induction of functional T cell hyporesponsiveness by the CD45RO/RB binding molecule, the antibody may be used in diseases, such autoimmune diseases, transplant rejection, psoriasis, inflammatory bowel disease or allergies, where an activated T cell population is thought to contribute to the pathology.

Example 12: In vivo studies in SCID-hu Skin mice

In this study, the utility of the CD45RB/RO binding chimeric antibody in a Psoriasis model system is tested. Human skin from normal individuals is transplanted to SCID (SCID-hu Skin) mice and the inflammatory process is mimicked by transferring mononuclear cells of unrelated donors into the SCID-hu Skin mice.

Transplantation of human adult skin in SCID mice (SCID-hu Skin mice)

Two small pieces (1 cm²) of human adult skin (obtained from the West Hungarian Regional Tissue Bank; WHRTB, Gyor) consisting of the entire epidermis, the papillary dermis and part of the reticular dermis, are transplanted at the right and left upper-back sides of SCID mice C.B 17 /GbmsTac-Prkdc^{scid} Lysf^{bg} mice (Taconic, Germantown, NY) in replacement of mouse skin. The quality of the grafts is monitored during 5-6 weeks following transplantation and successfully transplanted mice (SCID-hu Skin mice, generally >85%) are selected for in vivo testing of CD45RB/RO binding chimeric antibody.

Engraftment of human mononuclear cells in SCID mice

Mononuclear splenocytes (Spl) are isolated from human adult spleen biopsies (WHRTB, Gyor) after cell suspension (using a cell dissociation sieve equipped with a size 50 mesh) and standard density gradient procedures. Aliquots of ~5 x10⁸ Spl are re-suspended in 1.5 ml of RPMI-10% FCS and injected intraperitoneally (i.p.), on experimental day 0, into the SCID-hu Skin mice. These Spl numbers have been found in previous experiments to be sufficient to induce a lethal xeno-GvHD in >90% of the mice within 4-6 weeks after cell transfer.

Antibody treatment of SCID-hu Skin mice

SCID-hu Skin mice, reconstituted with human Spl, are treated with CD45RB/RO binding chimeric antibody or with anti-LPS control mAb at day 0, immediately after mononuclear cell injection, at days 3 and 7 and at weekly intervals thereafter. Antibodies are delivered subcutaneously (s.c.) in 100 µl PBS at a final concentration of 1 mg/kg body weight (b.w.).

Evaluation of anti-CD45 treatment

The efficacy of CD45RB/RO binding chimeric antibody is assessed by the survival of the transplanted mice and by monitoring the rejection of the skin grafts. The significance of the

results is evaluated by the statistical method of survival analysis using the Log-rank test (Mantel method) with the help of Systat v10 software. At the end of the experiment biopsies of human skin grafts and mouse liver, lung, kidney and spleen are obtained from sacrificed mice for histological purposes. All mice are weighed at the beginning (before cell transfer) and throughout the experiment (every two days) as an indirect estimation of their health status. Linear regression lines are generated using the body weight versus days post-PBMC transfer values obtained from each mouse and subsequently, their slopes (control versus anti-CD45 treated mice) are compared using the non parametric Mann-Whitney test.

10 *Results*

The human skin grafts are very well tolerated by the SCID mice. Initially, the grafts undergo a period of keratinocyte hyperproliferation resulting in the formation of hyperkeratotic crusts. About 5 weeks after transplantation, the crusts fall off the grafts and reveal a tissue containing all the characteristic structures observed in normal human skin. During this process, the human skin grafts fuse with the adjacent mouse skin and generate a network of freshly grown human vessels that connect the grafts with the underlying mouse tissue. The circulating human Spl transferred into SCID-hu Skin mice (at experimental day 0, approx. 6 weeks after skin transplantation) infiltrate the skin grafts and after recognition of alloantigen molecules expressed on the human skin mount an inflammatory response that in some cases completely destroy the graft.

Treatment of these mice with CD45RB/RO binding chimeric antibody suppresses the inflammatory process and prevents the rejection of the human skin grafts. In contrast, the sample obtained from the control treated mouse shows a massive infiltration with multiple signs of necrosis and a dramatic destruction of the epidermis. This process is easily monitored by eye and documented by simple photography of the mice.

Six out of six SCID-hu Skin mice transferred with allogeneic human Spl and treated with control anti-LPS mAb show a strong inflammatory response clearly visible by eye 23 days after mononuclear cell transfer. All mice show considerable lesions, including erythema, scaling and pronounced pustules. In contrast the skin grafts of all mice treated with CD45RB/RO binding chimeric antibody have a normal appearance. The dramatic differences between the two groups of mice is specifically due to the antibody treatment since the human skin of all mice have an identical look at the beginning of the experiment. This aspect

is not changed until the second week after cell transfer, the time at which the control group started to developed skin lesions. The experiment is terminated at day 34 after mononuclear cell transfer. By that time, one of the control mice is already dead (day 30) and four other are sacrificed (days 27, 27, 27 and 30) due to a strong xeno-GvHD. The pathologic reactions
5 observed in the antibody control treated mice also correlates with a loss of body weight in these animals.

In contrast, the CD45RB/RO binding chimeric antibody treated group displays a healthy status during the whole experimentation time.